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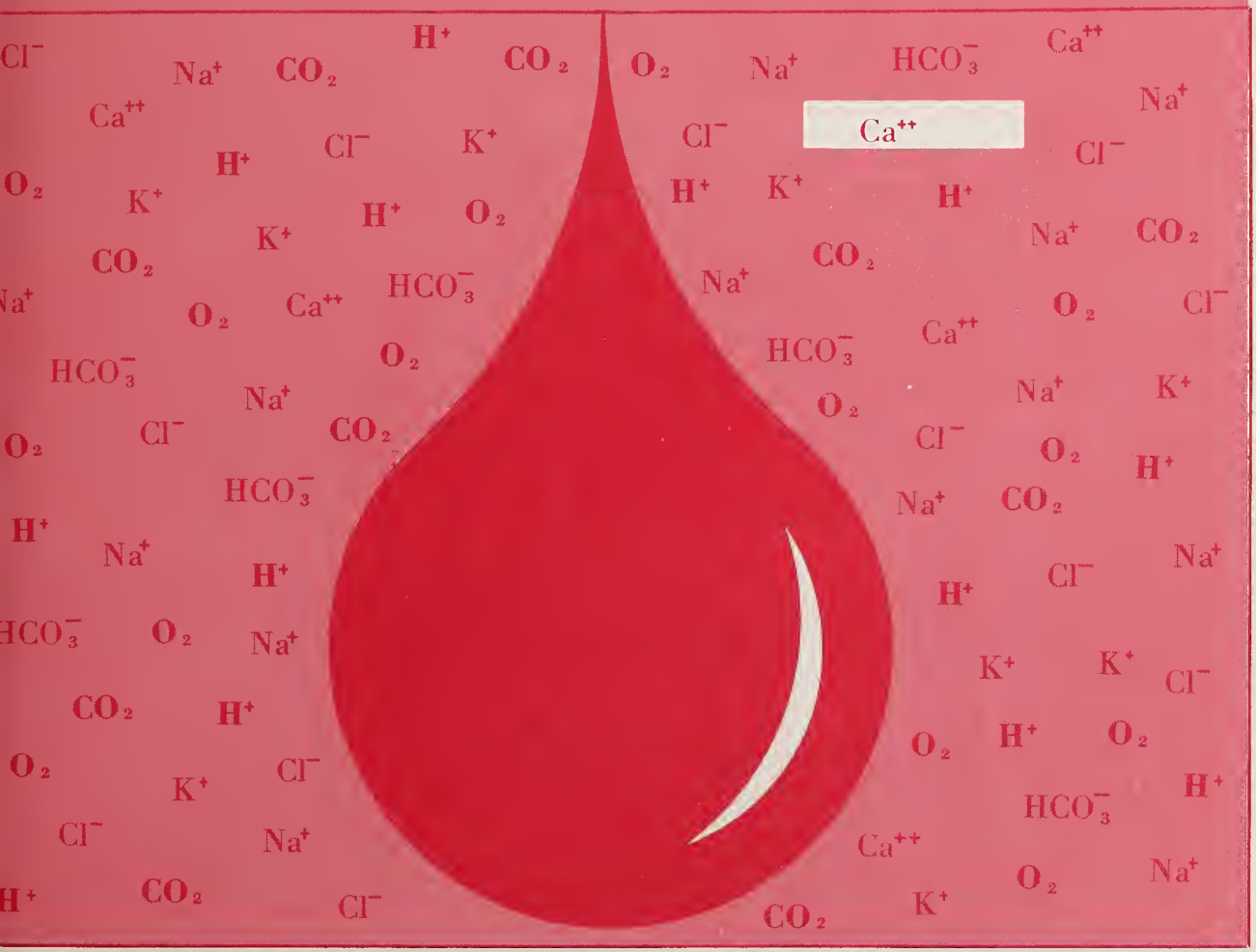
Blood pH, Gases, and Electrolytes



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[1] Headquarters and Laboratories at Gaithersburg, Maryland, unless otherwise noted; mailing address Washington, D.C. 20234.

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Blood pH, Gases, and Electrolytes

Proceedings of the workshop on
pH and Blood Gases, held at the
National Bureau of Standards,
Gaithersburg, Maryland,
July 7-8, 1975

Richard A. Durst, Editor

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FOREWORD

The Analytical Chemistry Division of the National Bureau of Standards (NBS), Institute for Materials Research has as its goal the development of new techniques of analysis, standard materials and instruments to improve the accuracy and precision with which analyses are performed. To obtain as broad input as possible from the user community on the needs and trends in analytical chemistry and to disseminate information outside NBS, groups of experts are invited to attend workshops at the Bureau. These workshops often are cosponsored by other scientific and professional organizations. These proceedings include the presentations and deliberations of one of a series of Analytical Chemistry Division sponsored workshops and are concerned with the accurate measurement of blood pH and gases. Other workshops in this series have included Secondary Ion Mass Spectrometry, Aerosol Measurements, Marine Pollution Monitoring (Petroleum), Monte Carlo Calculations in Ion Probe Microanalysis and Scanning Electron Microscopy, Standards for Spectrophotometry and Luminescence, Standards Required in Marine Science, Standards Required for Offshore Oil Drilling, and Standards Required for Environmental Monitoring of Oil Shale Processing.

The international character of the attendance at this workshop and of the interest exhibited by the cosponsoring organizations: the American Association of Clinical Chemists, the American Society of Clinical Pathologists, the International Federation of Clinical Chemistry, and the National Committee for Clinical Laboratory Standards, are indicative of the interest and importance of this subject throughout the clinical community. This publication should serve to characterize the current state of accuracy for blood pH and gases measurements and to point out the standards required in this field.

P. D. LaFleur
Chief
Analytical Chemistry Division

PREFACE

This publication is the formal report of the proceedings of the Workshop on pH and Blood Gases sponsored by the American Association of Clinical Chemists, American Society of Clinical Pathologists, International Federation of Clinical Chemistry, National Committee for Clinical Laboratory Standards, and the National Bureau of Standards. The purpose of this meeting was to discuss the status and needs in this very important area of clinical measurement and to provide a starting point for future cooperative efforts on an international level toward the standardization of pH and blood gas measurements and the various quantities and terms used in this field.

The importance of the acid-base status for clinical diagnosis is reflected in the fact that the determination of pH and blood gases is one of the most widely and frequently performed clinical tests. However, even though it has been more than a half century since Henderson and Van Slyke described the chemical and physiological relationships and mechanisms involved in acid-base balance, there still remain many uncertainties as to which indices are best measured, what parameters should be derived from the measured data and how to calculate them, and finally how these data should be interpreted by the clinicians.

The first requirement is an internationally agreed-upon set of definitions, terms, and symbols. The conflict of ideas that still exists is serious, not only because of the need for uniformity in nomenclature, but also for the more pragmatic reason that, as highly sophisticated blood pH/gas analyzers are developed, the derived parameters will be critically dependent upon the assumptions and "standard values" used in the calculations. It is hoped that this workshop and the resulting proceedings will provide a preliminary step toward achieving international accord in this area. Of course, a two-day workshop allowed little time for problem solving or the resolution of details. However, it did permit the exposure of diverse viewpoints on the critical needs in this field so that subsequent efforts, such as those of the newly established IFCC Expert Panel on pH and Blood Gases, can be directed toward the most important topics.

Editorial changes have been made to achieve some measure of internal consistency, especially with regard to the International System of Units (SI). However no changes are made in the nomenclature used by the authors since one of the purposes of this workshop was to reveal the diversity of symbols, terms, definitions, etc., in order to arrive at some future uniformity in this subject. Also, certain fundamental discussions appear in more than one paper but, again, in view of the diversity of approaches to this subject, this duplication was intentionally preserved. Although all the papers have been reviewed by the editor, the views expressed are entirely the responsibility of the individual authors.

In order to specify the procedures adequately, it has been necessary to identify commercial materials and equipment in this report. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment is necessarily the best available for the purpose.

The organization of the workshop and publication of these proceedings would not have been possible without the cooperation and assistance of a large group of people within the National Bureau of Standards. Particular thanks are given to Philip D. LaFleur, Chief of the Analytical Chemistry Division, for his encouragement and support in this effort, and to Ronald B. Johnson and Robert F. Martin of the Institute for Materials Research for their assistance in the business matters associated with this project. I also want to express my appreciation to Ellen N. Ring and her staff in the IMR Text-Editing Facility. The NBS Office of Information Activities, with special help from Sara R. Torrence, and also Rebecca J. Morehouse and Miriam K. Oland of the Office of Technical Publications gave invaluable assistance in many phases of the effort, varying from the initial meeting arrangements to the final publication of these proceedings. Special thanks are given to Gloria Burdick for her help in preparing the original workshop correspondence and program, Rosemary Hormuth for transcription of the recorded discussions, and Carolyn A. Shipley for her continuous effort in typing the coded manuscripts.

Richard A. Durst

ABSTRACT

On July 7-8, 1975, a workshop was held at the National Bureau of Standards to discuss the status and needs of this very important area of clinical measurement. A major goal of this workshop was the initiation of cooperative efforts on an international level toward the standardization of pH and blood gas measurements and the various quantities and terms used in this field.

To this end, the first technical session was concerned with the acid-base status of blood and included the topics: Definitions of Quantities and Concepts; Recommendations of Nomenclature, Physiological Terminology and Symbols; Reference Values; and the Evaluation of Nomograms and Algorithms. The second session addressed itself to the more practical aspects of this subject and included the topics: Blood Sampling, Handling, and Storage; Instrument Specifications; Quality Control and Standards; and the Development of Reference Methods. Finally, a brief session was held on the newer topic of the electrometric measurement of blood electrolytes.

This volume contains all of the papers invited for presentation at the workshop by some of the leading clinical and medical authorities on this subject and also includes a transcription of the extensive discussion sessions.

Key words: acid-base status; blood electrolytes; blood gases; blood pH; calcium; carbon dioxide; hydrogen ion concentration; nomograms; oxygen; P_{CO_2}; pH; P_{O_2}; potassium; sodium.

CONTENTS

National Bureau of Standards Special Publication 450. Proceedings of a Workshop on pH and Blood Gases held at NBS, Gaithersburg, Maryland, July 7-8, 1975. Issued June 1977.

DEFINITIONS OF ACID-BASE QUANTITIES: TERMINOLOGY, SYMBOLS, AND SI UNITS

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In clinical medicine, patients are often encountered with disturbances characterized by accumulation or loss of hydrogen ions and/or carbon dioxide. For routine clinical purposes, such disturbances are evaluated by measuring various quantities, for example, the "blood pH", the "hydrogen ion concentration", the "eucapnic pH", the "pCO_2", the "total-CO_2", the "plasma bicarbonate", the "CO_2 combining power", the "CO_2 capacity", the "standard bicarbonate", the "buffer base", and the "base excess." Most of these designations are "clinical slang" and they ought to be replaced by the more systematical chemical terminology which has been recommended by various international organizations [1-5][1].

1. Names and Symbols for Chemical Quantities

The name of a physical or chemical quantity must include a specification of the *kind of quantity* and a sufficiently detailed characterization of *the physical system* to which the quantity refers. This often involves a specification of one or more components of the system or processes or reactions in the system.

Table 1 shows the names of some of the acid-base quantities according to the international recommendations [2]. The names follow the scheme: System-component, kind of quantity. For example: P-hydrogen ion, substance concentration equals 53 nmol/l. In symbols this may be written [6]:

$$c\mathrm{H}^+(\mathrm{P}) = \{c\mathrm{H}^+(\mathrm{P})\} \cdot [c\mathrm{H}^+(\mathrm{P})] = 53\ \mathrm{nmol/l} \quad . \tag{1}$$

The name as well as the symbol should (explicitly or implicitly) include the following specifications:

(1) Kind of quantity. The different kinds of quantities are defined by the International Organization for Standardization in a publication series: ISO 31 [3]. The total number of different kinds of quantities amounts to several hundred. In clinical chemistry, the kinds of quantities listed in table 2 are of special interest. The symbol for the kind of quantity should always be a single letter and should always be printed in italics (sloping type), while all other symbols are printed in Roman type (upright) [1]. An exception to this rule is pH which unfortunately has been defined as a special kind of quantity (see later), but nevertheless is written in upright type.

According to the recommendations of the International Federation of Clinical Chemistry (IFCC) and the International Union of Pure and Applied Chemistry (IUPAC) [3,4], *substance concentration* (or simply *concentration*) should be employed rather than *mass concentration* in all cases where a formula unit can be defined for the component. For example, the hemoglobin concentration should be the substance concentration based on a molar mass, of 16114 g/mol referring to one quarter of a hemoglobin molecule with two α and two β chains.

[1]Figures in brackets indicate literature references at the end of this paper.

Table 1. Names of various acid-base quantities according to the recommendations of the International Federation of Clinical Chemistry (IFCC) and the International Union of Pure and Applied Chemistry (IUPAC) [2]. (The symbols are author's suggestions.)

Name	Symbol
U - Acid (H), substance concentration (method)	$\Delta c\mathrm{tH}^+(\mathrm{U})$
aB - Base (H^+-binding groups), substance concentration (Singer and Hastings, 1948)	$c\mathrm{BB}'(\mathrm{aB})$
aB - Base (H^+-binding groups), substance concentration difference (method; Pt - Norm)	$\Delta c\mathrm{B}'(\mathrm{aB})$
Gas (aB equil.) - Carbon dioxide, partial pressure (method; 37.0 °C)	$p\mathrm{CO}_2(\mathrm{aB})$
(aB) P - Carbon dioxide, substance concentration	$c\mathrm{CO}_2(\mathrm{P{\subset}aB})$
P - Carbonate + carbon dioxide, substance concentration	$c\mathrm{tCO}_2(\mathrm{P})$
P - Hydrogen carbonate ion, substance concentration (blood; $c(O_2)$ = 0.21 mmol/l; $c(CO_2)$ = 1.19 mmol/l; Θ = 37 °C)	$c\mathrm{HCO}_3^-(\mathrm{P{\subset}stB})$
(aB)P - Hydrogen ion, substance concentration	$c\mathrm{H}^+(\mathrm{P{\subset}aB})$
aB - Plasma, pH (37 °C)	$p a\mathrm{H}^+(\mathrm{P{\subset}B})$
Pt - Urine, pH	$p a\mathrm{H}^+(\mathrm{U})$

Table 2. Various kinds of quantities generally employed in clinical chemistry.

Name	Symbol	Unit	Definition (C = component, S = system)
Number	N	1 (one)	number of particles or molecules
Amount of substance	n	mol	number of formula units: $6.023 \cdot 10^{23} \cdot n\mathrm{C/mol} = N\mathrm{C}$
Mass	m	kg	
Volume	V	l (or m^3)	
Number fraction	X	1 (one)	$X\mathrm{C}_j(\mathrm{S}) = N\mathrm{C}_j(\mathrm{S})/\sum_i N\mathrm{C}_i(\mathrm{S})$
Substance fraction	x	1 (one)	$x\mathrm{C}_j(\mathrm{S}) = n\mathrm{C}_j(\mathrm{S})/\sum_i n\mathrm{C}_i(\mathrm{S})$
Mass fraction	w	1 (one)	$w\mathrm{C(S)} = m\mathrm{C(S)}/m\mathrm{S}$
Volume fraction	ϕ	1 (one)	$\phi\mathrm{C(S)} = V\mathrm{C(S)}/V\mathrm{S}$
Number concentration	C	l^{-1}	$C\mathrm{C(S)} = N\mathrm{C(S)}/V\mathrm{S}$
Substance concentration	c	mol/l	$c\mathrm{C(S)} = n\mathrm{C(S)}/V\mathrm{S}$
Mass concentration	ρ	kg/l	$\rho\mathrm{C(S)} = m\mathrm{C(S)}/V\mathrm{S}$
Molality	$\acute{m}$	mol/kg	$\acute{m}\mathrm{C(S)} = n\mathrm{C(S)}/m\mathrm{Solvent(S)}$
(Partial) pressure	p	Pa	$p\mathrm{C(S)} = x\mathrm{C(S)} \cdot p\mathrm{S}$

(2) Component. The names of the components are specified in considerable detail according to a certain set of rules [2]. Several of the names may be expressed in alternative forms, *e.g.*, carbonate + carbon dioxide = total CO_2. The symbols for the inorganic substances should be the familiar chemical symbols. For certain organic substances, symbols are also almost international, *e.g.*, hemoglobin = Hb, protein = Pr.

(3) System. The above mentioned recommendations include a list of symbols for the systems, *e.g.* blood = B, arterial blood = aB, plasma = P, urine = U, *etc.*

(4) Number. Braces {} around the symbol for the quantity indicate the pure number value of the quantity [1].

(5) Unit. Brackets [] around the symbol for the quantity indicate the unit of the quantity [1]. Whenever possible, it is recommended to use the SI units (m, kg, s, -system). This means that the traditional unit for pressure, mmHg, should be replaced by Pa (= N/m^2), conversion factor: 1 mmHg = 0.133 kPa.

The names recommended by IFCC and IUPAC are especially designed for tables and laboratory records [2]. In a running text or in the spoken language, a different order is often chosen, *e.g.*, the concentration of hydrogen ions in the plasma, the concentration of plasma hydrogen ion, the plasma hydrogen ion concentration, the plasma concentration of hydrogen ions, the hydrogen ion concentration in the plasma. All combinations are seen in the literature.

2. Stock Components, Amount of Substance, and Chemical Potential

The fundamental basis for a description of acid-base physiology was the development of physical chemistry and thermodynamics which led to an exact description of a chemical system at the beginning of this century.

The organism is an open chemical system in a dynamic equilibrium with its surroundings. A complete description of the system therefore requires not only a description of the momentary composition of the different body phases, including the *acid-base status* of the blood, but also requires a description of the rate of intake, production, conversion, and excretion of acids and bases, *i.e.*, a description of the *acid-base balance* of the organism.

A chemical system, *e.g.*, blood plasma, can theoretically be prepared from a limited number of stock components, for example, H_2O, Na_3PO_4, HCl, CO_2, O_2, NaCl, etc. It is not the purpose here to describe all the stock components of plasma. We are in the present connection especially interested in changes in the amount of hydrogen ions in the system.

Adding hydrogen ions to the system presents a special problem because they cannot be added or removed alone, according to the law of electrical neutrality. With the present choice of stock components, a hydrogen ion is always accompanied by a chloride ion. Alternatively, hydrogen ions could be added or removed in exchange for sodium ions which virtually means removing or adding NaOH. The first method is described as addition or removal of strong acid, the second method as removal or addition of strong base, and the two methods are considered equivalent, although the effects on the ionic strength will be slightly different.

The stock components can react mutually giving rise to several *derived* components, *e.g.*,

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- \quad . \tag{2}$$

with the above-mentioned choice of stock components, HCO_3^- is considered a derived component. However, the choice of stock components is arbitrary; one could also choose H_2O, $NaHCO_3$, and HCl as stock components, in which case CO_2 would be a derived component.

The first mentioned choice of stock components is based on physiological considerations, according to which H_2O, CO_2, and strong acid or base are ingested or formed in the organism and excreted in the lungs and kidneys.

The important equation which expresses the change in free energy of the system (dG) when the amounts of stock components are changed (at constant temperature and pressure) is the following:

$$\begin{aligned} dG = {} & \tilde{\mu}H^+ \cdot dntH^+ \\ & + \mu CO_2 \cdot dntCO_2 \\ & + \tilde{\mu}Cl^- \cdot dntCl^- \\ & + \tilde{\mu}Na^+ \cdot dntNa^+ \\ & + \mu O_2 \cdot dntO_2, \text{ etc.} \end{aligned} \qquad (3)$$

t = prefix designating total amount added,

n = amount of substance (unit: mole),

$\tilde{\mu}$ = electrochemical potential (unit: joule/mole),

$= \mu + z \cdot F \cdot \phi$,

where μ = chemical potential, z = charge number, F = Faraday constant, and ϕ = inner electrical potential.

This important equation forms the basis for derivation of important relationships among the various quantities. In the present connection, the equation only serves to define the *chemical potential* as the partial molar free energy of the different components, and the equation serves to emphasize that each stock component of the system can be characterized by two quantities: (1) an *intensive* quantity, *i.e.*, the chemical potential, and (2) an *extensive* quantity, *i.e.*, the amount of substance of added component.

The component of primary interest is H^+ (strong acid or base), but it is necessary to deal with another component which greatly influences the chemical potential of H^+ in an aqueous solution, namely CO_2. In the following, the intensive and extensive quantities are described in more detail for these two components.

3. Chemical Potential of Hydrogen Ions, $\Delta\mu H^+$ or pH

From a physico-chemical point of view, the chemical potential of the hydrogen ions is the most important quantity for description of the "acidity" of a solution. The significance of the chemical potential is that hydrogen ions tend to diffuse from a phase with a higher chemical potential to a phase with a lower potential, unless an electrical potential difference exists between the phases. In that case, the equilibrium condition requires that the electrochemical potential (*i.e.*, the sum of the chemical potential and the electrical potential times z times the Faraday constant) should be identical in the two phases.

It must be emphasized that it is impossible (theoretically) to measure absolute chemical potentials. Using two ideal H^+ electrodes: $E_L|S_L|KCl|S_R|E_R$, we can measure $\mu H^+(S_R) - \mu H^+(S_L) + F\Sigma\Delta E(LJ)$. It is possible to minimize $\Sigma\Delta E(LJ)$ by means of a saturated KCl bridge. If S_L is a reference solution (S^Θ), we can therefore measure (approximately):

$$\Delta\mu H^+(S^\Theta/S_R) \equiv \Delta_\Theta\mu H^+(S_R) = \mu H^+(S_R) - \mu H^+(S^\Theta) \quad , \qquad (4)$$

i.e., the excess chemical potential of hydrogen ions in solution S_R. The reference solution is chosen so that $a\mathrm{H}^+(S^\Theta) = 1$ (see below).

Often the activity of the hydrogen ions is used as a measure of the acidity. The activity is related to the chemical potential as follows:

$$a^*\mathrm{H}^+(S) = \exp \frac{\mu\mathrm{H}^+(S)}{RT} \tag{5}$$

$$r a^*\mathrm{H}^+(S^\Theta/S) \equiv r_\Theta a^*\mathrm{H}^+(S) \equiv \frac{a^*\mathrm{H}^+(S)}{a^*\mathrm{H}^+(S^\Theta)} \equiv a\mathrm{H}^+(S) \tag{6}$$

where a^* is absolute activity and a is relative activity; r is a convenient mathematical symbol for ratio (compare Δ: $\ln r x = \Delta \ln x$). The reference solution (S^Θ) is defined by the following equations which also define the activity coefficient γ:

$$a\mathrm{H}^+(S) = \frac{\gamma\mathrm{H}^+(S) \cdot \acute{m}\mathrm{H}^+(S)}{\acute{m}\mathrm{H}^+(S^\Theta)}\ ;\ x\mathrm{H_2O}(S) \to 1 \Rightarrow \gamma\mathrm{H}^+(S) \to 1 \quad . \tag{7}$$

S^Θ may be said to be a hypothetical ideal solution with $\acute{m}\mathrm{H}^+(S^\Theta) = 1$ mol/kg and $\gamma\mathrm{H}^+(S^\Theta) = 1$. Actually, $a\mathrm{H}^+$ is about 1 in a hydrochloric acid solution with $\acute{m}\mathrm{H}^+$ about 0.85 mol/kg, *i.e.*, $\gamma\mathrm{H}^+ = 1.18$. $\gamma\mathrm{H}^+$ varies with the ionic strength. For human plasma, $\gamma\mathrm{H}^+(P)$ is often assumed to be about 0.8.

Calling the mathematical operator -log for p, gives the familiar pH quantity:

$$-\log a\mathrm{H}^+ = p a\mathrm{H}^+ = \mathrm{pH} \quad . \tag{8}$$

It will be seen that changes in pH (with opposite sign) are directly proportional to changes in the chemical potential of the hydrogen ions.

A number of international standard buffers have been defined in order to enable reproducible pH measurements by means of electrodes, and the pH concept has thereby been given an international "operational definition." It was unfortunate, however, that IUPAC defined pH as a special derived kind of quantity [3].

Occasionally *the hydrogen ion concentration* ($c\mathrm{H}^+$) is used as a measure of the acidity. The relationship between $c\mathrm{H}^+$ and $\acute{m}\mathrm{H}^+$ (*i.e.*, molality) is the following:

$$c\mathrm{H}^+(S) = \acute{m}\mathrm{H}^+(S) \cdot \rho\mathrm{H_2O}(S) \quad , \tag{9}$$

where $\rho\mathrm{H_2O}(S)$ is the mass concentration of H_2O in the system (*e.g.*, for plasma: $\rho\mathrm{H_2O}(P) = 0.94$ kg/l). In the medical literature, opinion is divided as to whether pH or $c\mathrm{H}^+$ should be preferred as a measure of the acidity [6]. For "medical purposes," the $c\mathrm{H}^+$ of the plasma is generally calculated without distinguishing between molality and concentration and without

taking the activity coefficient into account: c_{H^+} = antilog(9 - pH) nmol/l. The correct equation would be:

$$c_{H^+}(P) = \frac{\rho_{H_2O}(P)/(kg/l)}{\gamma_{H^+}(P)} \cdot \text{antilog}(9 - pH)\ \text{nmol/l} \qquad (10)$$

Now that SI units are strongly recommended, it seems logical to use the SI unit joule also for the "pH quantity", *i.e.*, to use excess chemical potential of hydrogen ions. For human arterial blood plasma, the reference range would be:

$\Delta\mu_{H^+}$: -43.76 to -44.23 kJ/mol, and the extreme pathological range:

$\Delta\mu_{H^+}$: -40.37 to -46.31 kJ/mol, (corresponding to pH 7.37 to 7.45, and 6.80 to 7.80, respectively).

For conversion of pH values to $\Delta\mu_{H^+}$ and vice versa, the following equation applies:

$$\Delta\mu_{H^+} = -RT \cdot \ln 10 \cdot pH \quad , \qquad (11)$$

or for T = 310.15 K:

$$\Delta\mu_{H^+} = -5.937 \cdot pH\ \text{kJ/mol} \quad . \qquad (12)$$

4. Titratable Acid or Base; the Base Excess Concept, Δc_{tH^+} or $\Delta c_{B'}$

The amount of added hydrogen ions in the system (n_{tH^+}), *i.e.*, added strong acid, is an arbitrary quantity which requires a definition of the system before the addition. If the initial state of the system is defined, then the amount of added strong acid or base can be determined by back titration with strong base, respectively, strong acid. In other words, one can define and determine the difference (Δn_{tH^+}): the amount of total hydrogen ion in the system before titration minus the amount of total hydrogen ion in the system after titration. By dividing with the volume of the system, this extensive quantity can be expressed as an "excess concentration" of total hydrogen ion:

$$\Delta c_{tH^+}(S) = \Delta n_{tH^+}(S)/V_S \quad . \qquad (13)$$

In the usual terminology, this quantity with opposite sign is designated the excess concentration of base, or simply the "base excess":

$$\Delta c_{tH^+}(S) = -\Delta c_{B'}(S) \quad . \qquad (14)$$

The base excess concentration can also be expressed as being the concentration of titratable base minus the concentration of titratable acid. For plasma, the endpoint of titration is defined at pH = 7.40, p_{CO_2} = 40 mmHg (= 5.33 kPa), and t = 37 °C. For whole blood, the endpoint is the same, the pH value referring to the plasma phase. For urine, the

endpoint may be defined at pH = 7.40, pCO_2 = 0, pNH_3 = 0, and t = 37 °C; by this choice HCO_3^- is included as titratable base, while NH_4^+ is included as titratable acid.

For urine, the titratable acid or base is generally determined directly by titration. For blood or plasma, however, the quantity is generally determined indirectly by calculation from the directly measured pH and pCO_2 values [6].

It appears that the concentration of total hydrogen ion in the plasma is without practical significance, contrary to, for example, the concentration of total calcium ion in the plasma. The reason is that the solvent itself (H_2O) contains large amounts of hydrogen ions in bound form. Therefore, it is only relevant to determine changes or differences in the concentration of strong acid or base in the solution. Apart from this, however, there is no principal difference between the description of H^+ and Ca^{++}: both ions can be added to or removed from the system (together with an indifferent anion) and both are bound specifically by binding groups, which in the case the hydrogen ions, are called base groups.

It is only in recent years that the base excess concentration has been used routinely for description of the acid-base status of the blood. Originally, the bicarbonate concentration or the total CO_2 concentration was used as indicators of the accumulation of noncarbonic acid or base, *i.e.*, as measures of a metabolic acid-base disturbance. When dealing with a pure bicarbonate solution, the change in the bicarbonate concentration equals the change in the concentration of titratable acid or base, and the bicarbonate concentration is unaffected by changes in pCO_2; but in the presence of non-bicarbonate buffers, the bicarbonate concentration also changes during isolated changes in the pCO_2 even when the concentration of titratable acid or base is constant. In order to compensate for this effect, the pCO_2 was standardized to a normal value of 40 mmHg (= 5.33 kPa), and the concentration of total CO_2 or HCO_3^- of such standardized blood or plasma was designated "the plasma CO_2 combining power," "the CO_2 capacity of the blood," or "the standard bicarbonate." However, none of these quantities accurately reflect the accumulation of noncarbonic acid or base in the blood or plasma.

5. The Chemical Potential or the Partial Pressure of CO_2, $\Delta\mu CO_2$, or pCO_2

Generally, the partial pressure of carbon dioxide in a gas phase in equilibrium with the blood is used as a measure of the chemical potential of CO_2. Conversion of pCO_2 to μCO_2 or vice versa follows the equation

$$\Delta\mu CO_2 = RT \cdot \ln(pCO_2/101.3\ \text{kPa}) \quad , \tag{15}$$

or at T = 310.15 K:

$$\Delta\mu CO_2 = 5.937 \cdot \log(pCO_2/101.3\ \text{kPa})\ \text{kJ/mol} \quad . \tag{16}$$

To be exact, pCO_2 should be multiplied by a fugacity coefficient which can be taken to be 1 for pressures below 100 kPa. The reference system is a system in equilibrium with a hypothetical ideal gas phase with pCO_2 = 101.3 kPa.

For chemical substances, IUPAC has recommended the use of molecular quantities, *i.e.*, substance concentration rather than mass concentration. This recommendation might be extended to a recommendation of using the excess chemical potential rather than other quantities (activity, partial pressure, concentration of free component, etc.) which are presently employed as substitutes for the chemical potential.

For human arterial blood, the reference range for the excess chemical potential of CO_2 is: $\Delta\mu CO_2$: -8.1 to -7.4 kJ/mol, and the extreme pathological range is about: $\Delta\mu CO_2$; -11.2 to -5.2 kJ/mol, (corresponding to pCO_2 4.3 to 5.7 kPa and 1.3 to 13.3 kPa, respectively).

The partial pressure of CO_2 is related to the concentration of dissolved CO_2 according to Henry's law:

$$cCO_2 \equiv aCO_2 \cdot pCO_2 \ , \tag{17}$$

where aCO_2 is the solubility coefficient. Generally, cCO_2 includes the very low concentration of H_2CO_3. The solubility coefficient for normal plasma is 0.306 mmol $\cdot$ l^{-1} $\cdot$ $mmHg^{-1}$ at 37 °C. (or in SI units: 0.231 mmol/J) with a normal biological standard deviation of about 0.0004 mmol $\cdot$ l^{-1} $\cdot$ $mmHg^{-1}$. For lipemic plasma, however, the solubility coefficient may be considerably higher, up to about 0.33 mmol $\cdot$ l^{-1} $\cdot$ $mmHg^{-1}$ [6].

6. The Concentration of Total CO_2, $ctCO_2$

The extensive quantity expressing the amount of added CO_2 in the system is converted to the concentration of total CO_2 in the system by division with the volume of the system:

$$nCO_2(S)/VS = ctCO_2(S) \ . \tag{18}$$

The quantity has been and still is much employed for description of the acid-base status of the blood. The reason is, most of all, that the concentration of total CO_2 is easy to measure gasometrically by means of the Van Slyke apparatus, or automatically by means of Leonard Skegg's continuous flow analyzer. For clinical purposes, this quantity is generally used as a measure of a metabolic acid-base disturbance, *i.e.*, as an indirect measure of the excess concentration of titratable acid or base.

The *bicarbonate concentration* of the plasma is often calculated as the concentration of total CO_2 minus physically dissolved CO_2:

$$cHCO_3^-(P) = ctCO_2(P) - cCO_2(P) \ . \tag{19}$$

Therefore, the "bicarbonate" concentration generally includes carbonate and carbamate. The bicarbonate concentration of the plasma is one of the components of the electrolyte scheme (Gamble diagram) and it is used for calculation of the so-called anion deficit (undetermined anions):

$$cUA^- = cNA^+ - (cCl^- + cHCO_3^-) \ . \tag{20}$$

The concentration of undetermined anions is used as an indicator of an accumulation of organic anions (lactate, β-hydroxybuturate, *etc.*) in the plasma.

7. Conclusion

In the preceding sections, four fundamental acid-base variables have been described, relating to the two components H^+ and CO_2:

(1) the excess chemical potential of H^+, $\Delta\mu H^+$;

(2) the excess concentration of total H^+, ΔctH^+;

(3) the excess chemical potential of CO_2, $\Delta\mu CO_2$; and

(4) the concentration of total CO_2, $ctCO_2$.

Only two of the four variables are *independent variables*; the other two being *dependent variables*.

From a physiological point of view, $\Delta\mu CO_2$ and ΔctH^+ are the independent variables, because the system is an open system in equilibrium with a gas phase (the alveolar air) with a certain $\Delta\mu CO_2$ value. In such an open system, it is possible independently to alter the $\Delta\mu CO_2$ of the gas phase or to add various amounts of strong acid or base (add or remove H^+). Clinically speaking, $\Delta\mu CO_2$ is a parameter (*i.e.*, indicator) of the alveolar ventilation, while ΔctH^+ is a parameter of the non-respiratory acid-base balance.

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THE BUFFER VALUE OF PLASMA, ERYTHROCYTE FLUID AND WHOLE BLOOD

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The buffer value (β)[3] for hydrogen ions in a solution (S) is generally defined as the slope of the titration curve at a given pH value when titrating the solution with strong acid or base (B') [1][4]:

$$\beta H^+(S) = \frac{\partial c B'(S)}{\partial pH(S)} . \qquad (1)$$

The unit is mol/l, although in certain cases it may be advantageous to use molality (m):

$$\beta m H^+(S) = \partial m B'(S)/\partial pH(S), \text{ unit: mol/kg.} \qquad (2)$$

An alternative definition of buffer value is:

$$\beta^* C(S) = \frac{\partial c tC(S)}{\partial \mu C(S)}, \qquad (3)$$

where tC = total added component C. The SI unit is $mol^2 \cdot l^{-1} \cdot J^{-1}$. C may be H^+ or any other component, *e.g.*, O_2. Actually, the reciprocal value might be more relevant being the first partial differential coefficient of the function:

$$\Delta\mu C(S) = F(c tC(S), -, -, ---). \qquad (4)$$

The relationship between the two buffer values of eqs. (1) and (3) is:

$$\beta^* H^+(S) \cdot R \cdot T \cdot \ln 10 = \beta H^+(S). \qquad (5)$$

The buffer value of H^+ in a solution can be expressed as the sum of the buffer values of the solvent (H_2O) and the solutes (C). The change in concentration of added base can be expressed as:

$$dcB'(S) = dcOH^-(S) - dcH^+(S) + \Sigma dcC'(S), \qquad (6)$$

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[3]Concerning terminology and symbols, the reader is referred to the preceding paper. c = concentration, μ = chemical potential.
[4]Figures in brackets indicate the literature references at the end of this paper.

where C' symbolizes a base group. Dividing with dpH gives:

$$\beta H^+(S) = \beta H^+(H_2O \subset S) + \Sigma \beta H^+(C \subset S). \tag{7}$$

The buffer value of the water is approximately:

$$\beta H^+(H_2O \subset S) \approx (c H^+(S) + c OH^-(S)) \cdot \ln 10. \tag{8}$$

Therefore the buffer value of the water can generally be ignored for $3 < pH < 11$.

The buffer value for H^+ due to solute $C(CH \rightleftarrows H^+ + C^-)$ is equal to:

$$\beta H^+(C \subset S) = \frac{\partial c C^-(S)}{\partial pH(S)} = - \frac{\partial c CH(S)}{\partial pH(S)} . \tag{9}$$

The equality is only valid for $cC = cC^- + cCH$ = constant. If the system is an open system where CH or C^- disappears, the amount which has disappeared must be included in order to obtain equality. This especially applies to the CO_2 and NH_3 buffers.

The buffer value of the solutes may be expressed as the molar buffer value (β_m) or as the specific buffer value (β_w):

$$\beta_m H^+(C \subseteq S) \equiv \beta H^+(C \subseteq S)/cC(S), \tag{10}$$

$$\beta_w H^+(C \subset S) \equiv \beta H^+(C \subset S)/\rho C(S), \tag{11}$$

where $\rho C(S)$ is the mass concentration of C in S.

The buffer value is a partial differential and the other independent variables which are maintained constant must be specified. The buffer value of a pure bicarbonate solution (S) is widely different for a closed system with constant $ctCO_2$ and for an open system with constant pCO_2. The following equations are obtained by differentiation of $K_{acA} = aH \cdot cHCO_3^-/(ctCO_2 - cHCO_3^-)$:

$$\begin{aligned} \beta H^+(S, \text{closed}) &= \left(\frac{\partial cB'(S)}{\partial pH(S)}\right)_{ctCO_2(S)} \\ &= \left(\frac{\partial cHCO_3^-(S)}{\partial pH(S)}\right)_{ctCO_2(S)} \\ &= 2.303\, ctCO_2 \frac{K_{acA}\ aH^+}{(K_{acA} + aH^+)^2} , \end{aligned} \tag{12}$$

$$\begin{aligned} \beta H^+(S, \text{open}) &= \left(\frac{\partial cB'(S)}{\partial pH(S)}\right)_{pCO_2(S)} \\ &= \left(\frac{\partial cHCO_3^-(S)}{\partial pH(S)}\right)_{pCO_2(S)} \\ &= 2.303 \cdot cHCO_3^-(S). \end{aligned} \tag{13}$$

The maximal molar buffer value for a closed system is therefore 0.576 for pH = $pK_{ac}A$. For pK = 6.1, pH = 7.4, c_tCO_2 = 25.7 mmol/l, and $c_{HCO_3^-}$ = 24.5 mmol/l, the values are: βH^+(S,closed) = 2.7 mmol/l, and βH^+(S, open) = 56.6 mmol/l. This illustrates the insignificance of the carbonic acid/bicarbonate buffer in a closed system at pH = 7.4, but the great importance in an open system [2].

1. Plasma

The buffer value for H^+ in plasma can be expressed as the sum of the buffer values of the bicarbonate buffer and the non-bicarbonate buffers. The change in the concentration of added base can be expressed as the following sum (ignoring water, compare eq. (6)):

$$dc_B'(P) = dc_{HCO_3^-}(P) + dc_{X'}(P). \tag{14}$$

Dividing with dpH gives:

$$\beta H^+(P) = \beta H^+(HCO_3^- \subset P) + \beta H^+(X \subset P). \tag{15}$$

In the following, X is used as a symbol for "nonbicarbonate buffer."

The buffer value of the nonbicarbonate buffers can be determined as the slope of the titration curve, when titrating the plasma in the absence of CO_2. The value for normal plasma ($\rho Pr(P)$ = 70 g/l, wAlb(Pr) = 0.60, t = 310.15 K) determined by Siggaard-Andersen and Rørth (unpublished experiments), shows a maximum of 7.8 mmol/l at pH = 7.3, while the value for pH = 7.40 is 7.7 mmol/l. The pH variation of β is shown in figure 1.

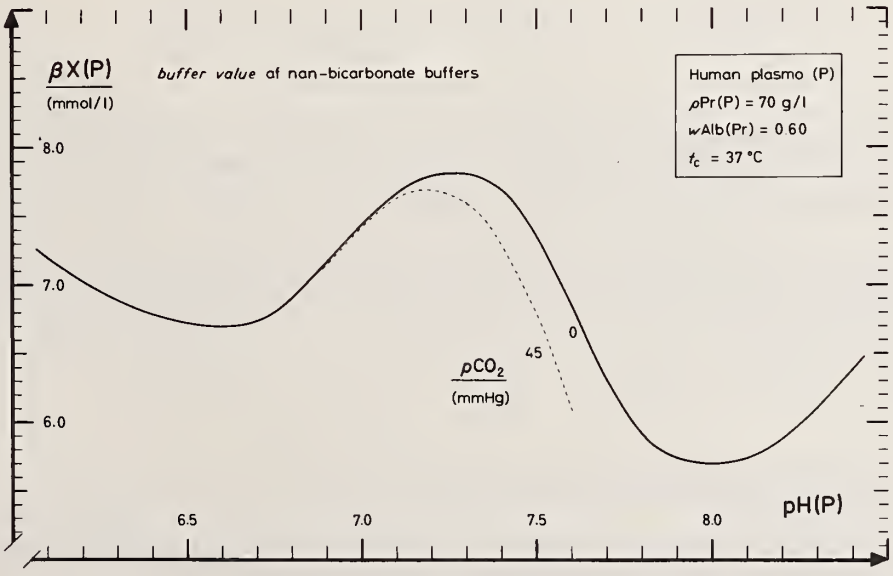

Figure 1. The buffer value for H^+ due to nonbicarbonate buffers in the plasma (symbol βH^+ (X ⊂ P) in the text) as a function of pH, in the absence of CO_2 as well as at $pCO_2 \approx 45$ mmHg [2]. ρ = mass concentration, w = mass fraction. The maximum at pH ≈ 7.3 is probably due to the imidazole groups of the albumin. The decrease in buffer value for pH > 7 in the presence of CO_2 is due to a change in pK value of the terminal amino groups from about 7.8 ($Pr\text{-}NH_3^+$) to about 5 (Pr-NH-COOH).

An alternative method for calculating the buffer value of the nonbicarbonate buffers of plasma is based on CO_2 titration. The reaction following CO_2 titration is:

$$X' + CO_2 + H_2O \rightarrow XH^+ + HCO_3^-, \tag{16}$$

where X' represents nonbicarbonate buffer base. To be more exact, HCO_3^- includes CO_3^- and $PrNHCOO^-$ and is therefore designated $tHCO_3^-$ = titratable bicarbonate. For varying pCO_2 we therefore have:

$$\left(\frac{\partial c tHCO_3^-(P)}{\partial pH(P)}\right)_{cB'(P)} = -\left(\frac{\partial cX'(P)}{\partial pH(P)}\right)_{cB'(P)} \tag{17}$$

As a good approximation we have:

$$\left(\frac{\partial cX'(P)}{\partial pH(P)}\right)_{cB'(P)} = \left(\frac{\partial cX'(P)}{\partial pH(P)}\right)_{pCO_2 = 0} \tag{18}$$

The first coefficient refers to varying $pCO_2(P)$ and the second to varying $\Delta cB'(P)$. The latter value should be slightly higher than the former in the pH range about pH = 8 (due to buffering by terminal amino groups), while the former should be slightly higher in the pH range about pH = 5 (due to buffering by the carbamate groups, $PrNHCOO^-$).

The coefficient $(\partial c tHCO_3^-/\partial pH)_{cB'}$ can be calculated from experimental values of $(\partial \log pCO_2/\partial pH)_{cB'}$:

$$\frac{d\log pCO_2}{dpH} = -(1 - dpK/dpH) + \frac{1}{2.3\ c tHCO_3} \cdot \frac{dc tHCO_3^-}{dpH} \quad . \tag{19}$$

This equation is obtained by differentiating $K = \alpha H^+ \cdot c tHCO_3^-/\alpha \cdot pCO_2$, taking α to be constant. $(\partial \log pCO_2(P)/\partial pH(P))_{cB'(P)}$ read on the curve nomogram provided the data for the buffer value of nonbicarbonate buffers which were plotted in figure 1 (dotted curve).

According to figure 1 the buffer value of the nonbicarbonate buffers of plasma varies significantly with pH. As an approximation the value is generally taken to be constant in the physiological pH range, independent of pCO_2 and pH, varying with the protein concentration only:

$$\beta H^+(X \subset P) = \rho Pr(P) \cdot 0.11\ mol/kg. \tag{20}$$

For $\rho Pr(P)$ = 70 g/l the buffer value therefore is 7.7 mmol/l.

Inorganic phosphate accounts for about 5 percent of the nonbicarbonate buffer value in normal plasma at pH = 7.4 (calculated from an equation anaolgous to eq. (12) with a total phosphate concentration of 1 mmol/l and $pK_{ac}A$ = 6.8).

Albumin is the major contributor to the buffer value of nonbicarbonate buffers of plasma. The imidazoles (16 per albumin molecule) are the most important buffer groups in the physiological pH range. Since the maximum buffer value in our experiments is at pH = 7.3, we assume that most of the imidazoles have dissociation constants of about $10^{-7.3}$. With an albumin concentration of $cAlb(P)$ = 0.62 mmol/l ($\Leftrightarrow \rho$ Alb(P) = 42 g/l) and hence an

imidazole concentration of 16 · 0.62⇒9.9 mmol/l, the maximal buffer value of the imidazoles at pH = 7.3 is calculated to be 0.576 · 9.9⇒5.7 mmol/l, *i.e.*, they account for 73 percent of the nonbicarbonate buffer value of plasma of 7.8 mmol/l at pH = 7.3. The remainder, *i.e.*, 22 percent ⇒ 1.7 mmol/l may be ascribed to the globulins, and with ρGlo(P) = 28 g/l, the specific buffer value of the globulins is calculated to be 0.06 mol/kg, while the specific buffer value of the albumin is 0.14 mol/kg.

2. Erythrocyte Fluid

The buffer value of the non-bicarbonate buffers of erythrocyte fluid determined by titrating erythrocyte fluid with strong base in the absence of CO_2 is shown in figure 2.

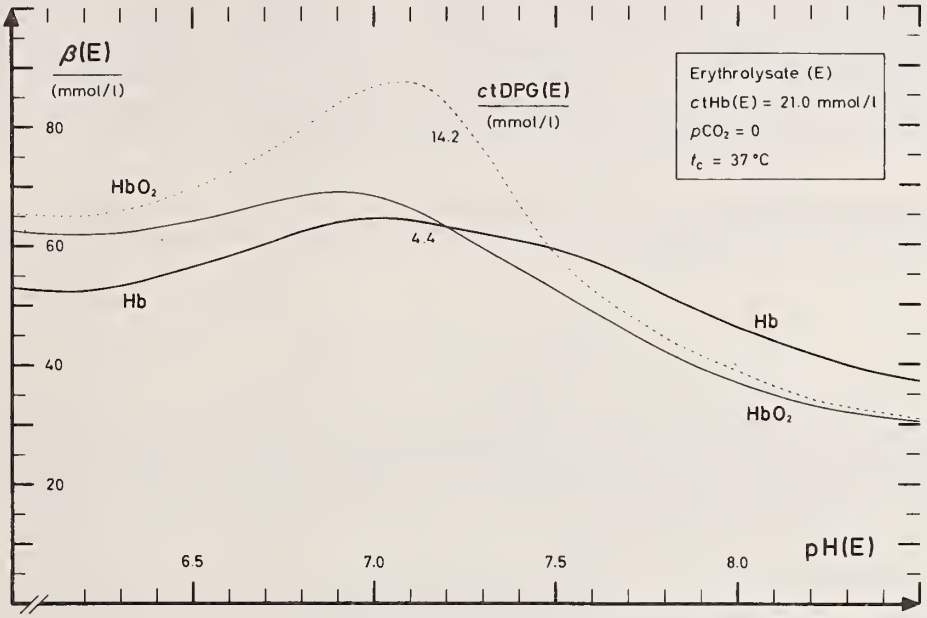

Figure 2. The buffer value for H^+ in erythrocyte fluid (symbol $\beta H^+(E)$ in the text) at pCO_2 = 0, *i.e.*, the buffer value for H^+ by non-bicarbonate buffers (symbol $\beta H^+(X \subset E)$ in the text), as a function of pH at 37 °C [2]. Hb = deoxyhemoglobin, HbO_2 = oxyhemoglobin. The dotted curve represents the buffer value of oxygenated erythrocyte fluid with a three-fold increase in 2,3-diphosphoglycerate concentration (*c*tDPG). The peak in the buffer value at pH = 7.1 indicates the p*K* value of some of the acid-base groups of the DPG molecule. The difference between the buffer value of oxygenated and deoxygenated erythrocyte fluid is due to the fall in p*K* value of the so-called oxygen-linked acid-base groups following oxygenation.

The value varies with pH, with the concentration of hemoglobin, with the oxygenation of the hemoglobin, and with the concentration of 2,3-diphosphoglycerate. The value for normal erythrocyte fluid is about 63 mmol/l for pH = 7.2, *c*Hb = 21 mmol/l, and *c*tDPG = 4 mmol/l [2].

2,3-DPG accounts for about 7 to 8 percent of this value. This is calculated from p*K* = 7.1 of two of the phosphate groups of DPG (by means of an equation analogous to eq. (12)) ⇒$\beta H^+(DPG \subset E) \approx 4.6$ mmol/l.

The remainder is mainly due to hemoglobin. The molar buffer value of the hemoglobin therefore is about 58/21 = 2.8. Assuming that the principal buffer groups in the physiological pH range are the imidazole groups, a maximum of about 6 imidazoles out of the 9.5 imidazoles of the hemoglobin chains (10 for α chains, 9 for β chains) are engaged in buffering at pH ≈ 7.2. The remainder may be engaged in salt bridge formation.

The difference between the buffer value of oxyhemoglobin and deoxyhemoglobin may be ascribed to the change in p*K* of the so-called oxygen-linked acid-base groups, *i.e.*, acid-

base groups which participate in salt bridges in deoxyhemoglobin but not in oxyhemoglobin. These salt bridges are the following, including those obtained by interchanging α_1 with α_2 and β_1 with β_2 [3]:

1) α-carboxyl of arginine HC3(141α_1) - α-amino of valine NA2(1α_2),

2) guanidinium of arginine HC3(141α_1) - β-carboxyl of aspartate H9(126α_2),

3) ε-amino of lysine C5(40α_1) - α-carboxyl of histidine HC3(146β_2),

4) imidazole of histidine HC3(146β_1) - β-carboxyl of aspartate FGI(94β_1).

The salt bridge formation causes a rise in pK of the C-terminal imidazole groups (HC3(146β)) and of the N-terminal amino groups (NA2(1α)) so that the buffer value of deoxyhemoglobin becomes higher than that of oxygemoglobin for pH > 7.2, but lower for pH < 7.2. The pK values are apparently shifted from the range of 7.8 to about 6.3. The greatest difference between the molar buffer value for oxyhemoglobin and deoxyhemoglobin (about 0.4 at pH 7.3 and 6.3) is less than the maximal difference (0.576) which should be expected if one buffer group per heme is shifted. The explanation may be that the pK values of the imidazoles and the valines differ somewhat so that the effect is smoothed.

As a rough approximation, the buffer value of the nonbicarbonate buffers of erythrocyte fluid may be taken to be independent of pH varying with the total hemoglobin concentration only [2]:

$$\beta H^+(X \subset E) = 3.0 \cdot c\mathrm{Hb}(E). \tag{21}$$

More accurate data for the buffer value of the nonbicarbonate buffers of plasma and erythrocyte fluid are necessary in order to improve the accuracy of the algorithms for calculating the relationship among the acid-base variables of blood and plasma (see subsequent paper on acid-base algorithms).

3. Whole Blood

The buffer value is a quantity which may refer to an equilibrium system of several phases, *e.g.* whole blood, provided we define the buffer value as:

$$\beta^* H^+(B) = \frac{\partial c_t H^+(B)}{\partial \tilde{\mu} H^+(B)}, \tag{22}$$

where $\tilde{\mu}$ is the electrochemical potential: $\tilde{\mu} = \mu + zF\Phi$, where F is the faraday constant (F = 96487 C/mol), and Φ is the inner electrical potential of the plasma. If the electrical potential of the continuous phase, *i.e.*, plasma, is the reference electrical potential, the buffer value of whole blood in terms of pH is:

$$\beta H^+(B) = \frac{\partial c_{B'}(B)}{\partial \mathrm{pH}(P)}. \tag{23}$$

The buffer value of the nonbicarbonate buffers of whole blood can be expressed in terms of the values for plasma and erythrocyte fluid. We have:

$$c_{X'}(B) = \phi P(B) \cdot c_{X'}(P) + \phi E(B) \cdot c_{X'}(E), \tag{24}$$

where $\phi P(B) + \phi E(B) = 1$. Differentiation of this equation with respect to pH, and rearrangement (regarding $\phi E(B)$ as constant) gives:

$$\frac{dcX'(B)}{dpH(P)} = \frac{dcX'(P)}{dpH(P)} + \phi E(B)\left(\frac{dcX'(E)}{dpH(E)} \cdot \frac{dpH(E)}{dpH(P)} - \frac{dcX'(P)}{dpH(P)}\right), \tag{25}$$

$$\beta H^+(X \subset B) = \beta H^+(X \subset P) + \phi E(B) \cdot (\beta H^+(X \subset E) \cdot \frac{dpH(E)}{dpH(P)} - \beta H^+(X \subset P)). \tag{26}$$

The relationship between pH(P) and pH(E) is given by (see eq. (6) of the subsequent paper) [4]:

$$pH(E) = 7.19 + 0.77\ (pH(P) - 7.40), \tag{27}$$

for oxygenated blood at 37 °C. Inserting $dpH(E)/dpH(P) = 0.77$ as well as $\beta H^+(X \subset P) = 7.7$ mmol/l and $\beta H^+(X \subset E) = 63$ mmol/l (for cHb(E) = 21 mmol/l) into eq. (26) gives:

$$\beta H^+(X \subset B) = (7.7 + \phi E(B) \cdot 40.8)\ \text{mmol/l}, \tag{28}$$

$$\beta H^+(X \subset B) = 1.94 \cdot c\text{Hb}(B) + 7.7\ \text{mmol/l}. \tag{29}$$

In analogy with eq. (17) we have:

$$\beta H^+(X \subset B) = -\left(\frac{\partial c tHCO_3^-(B)}{\partial pH(P)}\right)_{cB'(B)}, \tag{30}$$

where the concentration of bicarbonate refers to whole blood.

Often $(\partial c tHCO_3^-(P)/\partial pH(P))_{cB'(B)}$ is used as an indicator of the buffer value of non-bicarbonate buffers in the blood, where the concentration of bicarbonate refers to the plasma phase. We have:

$$\begin{aligned} c tHCO_3^-(B) &= c tHCO_3^-(P) \cdot \phi P(B) + c tHCO_3^-(E) \cdot \phi E(B) \\ &= c tHCO_3^-(P) \cdot (1 - (1 - rc) \cdot \phi E(B)), \end{aligned} \tag{31}$$

where $\phi P(B) + \phi E(B) = 1$, and $rcHCO_3^-(P|E) = c tHCO_3^-(E)/c tHCO_3^-(P)$. Regarding $\phi E(B)$ as constant, differentiation with respect to pH(P) and rearrangement gives:

$$-\frac{\partial c tHCO_3^-(P)}{\partial pH(P)} = \frac{-\frac{\partial c tHCO_3^-(B)}{\partial pH(P)} + \phi E(B) \cdot c tHCO_3^-(P) \cdot \frac{drc}{dpH(P)}}{(1 - (1 - rc) \cdot \phi E(B)} \tag{32}$$

By application of $1/(1-x) = 1 + x + x^2 + \ldots$ (for $|x| < 1$), we obtain:

$$\begin{aligned} -\left(\frac{\partial c tHCO_3^-(P)}{\partial pH(P)}\right)_{cB'(B)} = &\left(\beta H^+(X \subset B) + \phi E(B) \cdot c tHCO_3^-(P) \cdot \frac{drc}{dpH(P)}\right) \cdot \\ &(1 + (1-rc) \cdot \phi E(B) + ((1-rc) \cdot \phi E(B))^2 + \cdots\cdots). \end{aligned} \tag{33}$$

Inserting $\beta H^+(X \subset B)$ from eq. (26) we obtain:

$$-\left(\frac{\partial c tHCO_3^-(P)}{\partial pH(P)}\right)_{cB'(B)} = \beta H^+(X \subset P) + A \cdot \phi E(B) + A\,(1-rc) \cdot (\phi E(B))^2 + A\,(1-rc)^2 \cdot (\phi E(B))^3 + \ldots\ldots, \quad (34)$$

where:

$$A = \beta H^+(X \subset E) \cdot \frac{dpH(E)}{dpH(P)} - \beta H^+(X \subset P) \cdot r c + c tHCO_3^-(P) \cdot \frac{dr c}{dpH(P)} . \quad (35)$$

We assume that the small diffusible ions are at equilibrium across the red cell membrane and hence:

$$r a = \frac{a H^+(P)}{a H^+(E)} = \frac{a Cl^-(E)}{a Cl^-(P)} = \frac{a HCO_3^-(E)}{a HCO_3^-(P)} . \quad (36)$$

Conversion of $r a HCO_3^-$ to $r c HCO_3^- = c HCO_3^-(E)/c HCO_3^-(P)$ requires a knowledge of the ratio of the activity coefficients (γ) and the mass concentrations of H_2O (ρ):

$$r c HCO_3^-(P/E) = r a HCO_3^-(P/E) \cdot \frac{\gamma HCO_3^-(P)}{\gamma HCO_3^-(E)} \cdot \frac{\rho H_2O(E)}{\rho H_2O(P)} . \quad (37)$$

Experimental data indicate that $\gamma Cl^-(E)/\gamma Cl^-(P) = 0.93$ [1,3]. Using this value for $\gamma HCO_3^-(E)/\gamma HCO_3^-(P)$ and using $\rho H_2O(P) = 0.94$ kg/l, and $\rho H_2O(E) = 0.73$ kg/l, we have:

$$r c HCO_3^- = r a HCO_3^- \cdot 0.835. \quad (38)$$

From eq. (27) we obtain $dpH(E)/dpH(P) = 0.77$, and for pH = 7.40 we further obtain $r\alpha = 0.615$ and $dr\alpha/dpH(P) = -2.303\,(1-0.77)\,r\alpha = -0.326$. Hence $rc = 0.515$ and $drc/dpH(P) = 0.272$. Inserting these values together with $c tHCO_2^-(P) = 24.4$ mmol/l into eq. (35), we obtain: $A = 37.9$ mmol/l. As an approximation ignoring the variation with $c tHCO_3^-(P)$ and with pH(P) we therefore have:

$$-\left(\frac{\partial c HCO_3^-(P)}{\partial pH(P)}\right)_{cB'(B)} /(\text{mmol/l}) = 7.7 + 37.9\ \phi E(B) + 18.4\ (\phi E(B))^2 + 8.9\ (\phi E(B))^3 + \ldots . \quad (39)$$

As a further approximation fitting this equation for $\phi E(B) = 0$ and $\phi E(B) = 0.45$ and for $c HB(E) = 21$ mmol/l we get [2]:

$$-\left(\frac{\partial c HCO_3^-(P)}{\partial pH(P)}\right)_{cB'(B)} /(\text{mmol/l}) = 7.7 + 48.4\ \ \phi E(B) \quad (40)$$

$$= 7.7 + 2.3\ c Hb(B)/(\text{mmol/l}). \quad (41)$$

This is the approximation which is often employed for the slope of the pH, $c tHCO_3^-$ equilibration curve of whole blood. The present derivation indicates where the principal approximations have been made: (1) assuming the buffer values of nonbicarbonate buffers of plasma and erythrocyte fluid to be independent of pH, (2) ignoring the variation with $c tHCO_3^-$ at constant

pH(P), (3) ignoring the variation with pH(P) at constant $\sigma tHCO_3^-$ (P), and (4) assuming a linear variation with $\phi E(B)$.

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ACID-BASE ALGORITHMS

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A knowledge of the relationships between the acid-base variables of the blood *in vitro* is of considerable importance, because it is often required to calculate the remaining variables on the basis of measurement of only two of the variables. For these calculations various nomograms have generally been employed, *e.g.*, the Van Slyke and Sendroy nomogram [1][1], McLean's Z-nomogram [2], the Singer and Hastings nomogram [3], and others [4,5]. With the development of small programmable calculators, an arithmetic calculation will probably in the future be more suitable. The problem will often be that one wishes to calculate the base excess concentration of the blood, or perhaps of the average extracellular fluid, on the basis of pH and pCO_2 values measured at 37 °C. Process diagrams for two arithmetic algorithms for this purpose are shown in figures 1 and 2. The rectangular blocks are procedure symbols [6] and each of these will be described separately in the following. The word algorithm (or algorism) is derived from al Kuwarizmi, a Persian mathematician from the 8th century.

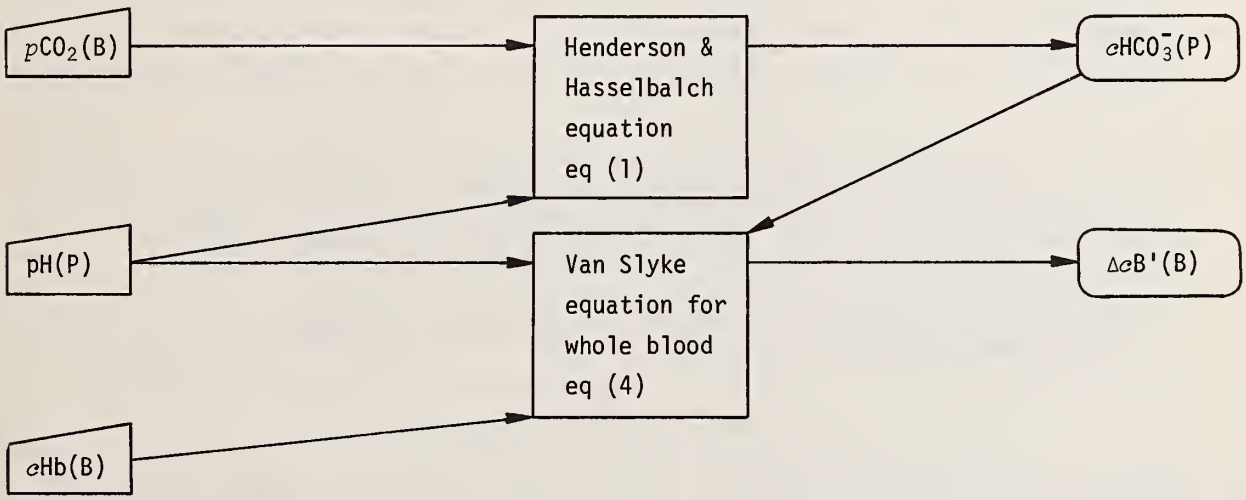

Figure 1. Procedure diagram for calculation of the bicarbonate concentration of the plasma, $cHCO_3^-(P)$ and the base excess concentration of blood or extracellular fluid $\Delta cB'(B)$ or $\Delta cB'(Ecf)$ after measuring the blood pCO_2, the plasma pH, and the hemoglobin concentration of the blood $cHb(B)$. The Henderson-Hasselbalch equation is employed for calculation of the plasma bicarbonate concentration. The Van Slyke equation for whole blood is employed for calculation of the base excess concentration. The various equations are described in the text.

[1] Figures in brackets indicate the literature references at the end of this paper.

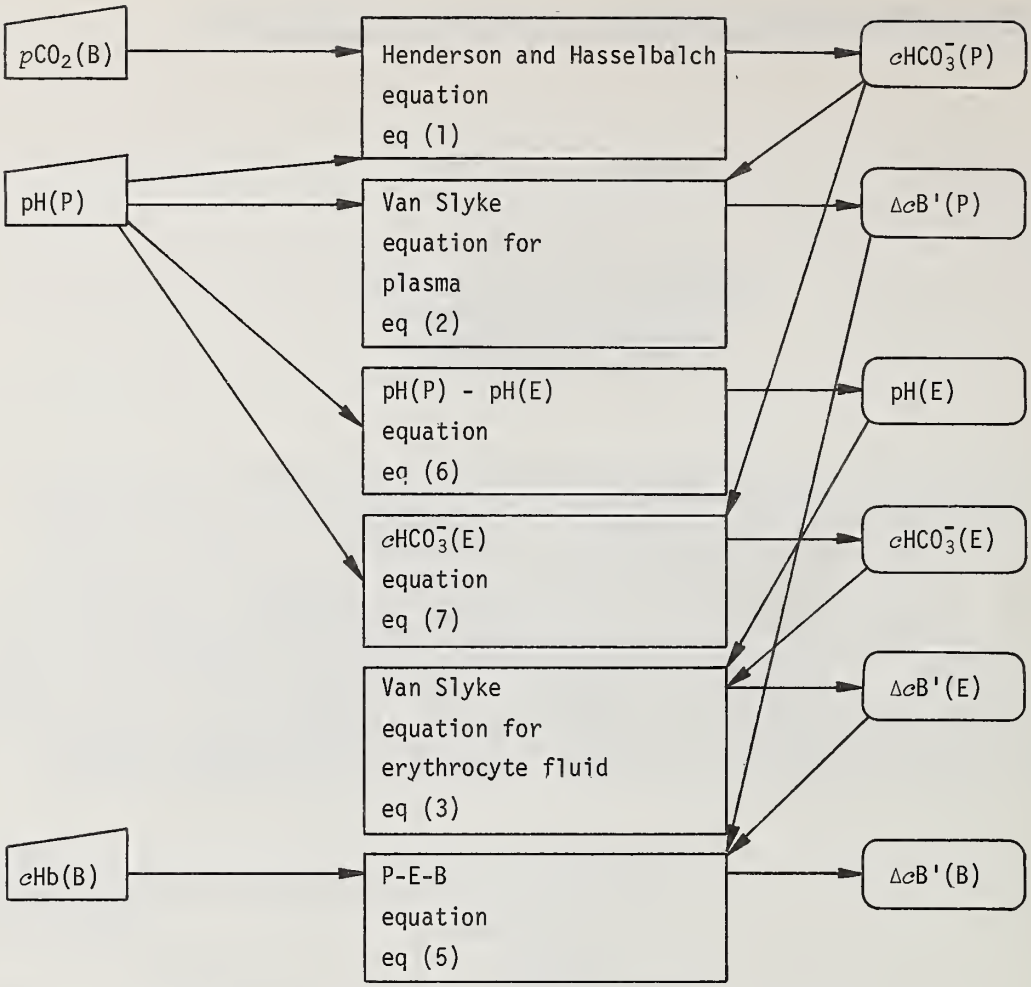

Figure 2. Alternative algorithm based on intermediary calculation of the pH of the erythrocyte fluid (pH(E)) and the bicarbonate concentration of the erythrocyte fluid ($cHCO_3^-$(E)). The Van Slyke equation for plasma respectively erythrocyte fluid is used for calculation of the base excess concentration of the plasma ($\Delta cB'$(B)) respectively erythrocyte fluid ($\Delta cB'$(E)). The various equations are described in the text. Another almost identical algorithm uses the Henderson-Hasselbalch equation for erythrocyte fluid to calculate $cHCO_3^-$(E) from pH(E) and pCO_2(B).

1. The Henderson-Hasselbalch Equation

The first equation of the algorithms is the Henderson-Hasselbalch equation, which can be expressed as follows for human plasma at 37 °C:[2]

$$pH = pK + \log \frac{cHCO_3^-}{\alpha CO_2 \cdot pCO_2} \,. \qquad (1)$$

$$pK = 6.10.$$

$$\alpha CO_2 = 0.0306 \text{ mmol/l} \cdot \text{mmHg} \; (= 0.231 \text{ mmol/l} \cdot \text{kPa}) \,.$$

[2] Concerning terminology and symbols the reader is referred to the preceding paper on definitions of acid-base quantities. HCO_3^- includes CO_3^- and carbamate throughout.

The value for pK varies slightly with the composition of the plasma, with a normal biological standard deviation of about 0.0015. In pathological cases with large changes in the ionic strength the variation is considerably greater. Due to the incorporation of carbonate and carbamate in the "bicarbonate" concentration an apparent variation of pK with pH appears (decreasing pK with increasing pH), but for most practical purposes the variation of pK can be ignored. The Henderson-Hasselbalch equation can be derived by expressing the equality of the sum of the chemical potentials of CO_2 and H_2O and the sum of the chemical potentials of H^+ and HCO_3^-.

2. The Van Slyke Equation

The CO_2 equilibration curve of the blood can, as a good approximation, be represented by a straight line in a pH, log pCO_2 coordinate system, and this is employed in the nomographic algorithms. Another good approximation to a straight line is obtained by plotting the CO_2 equilibration line in a pH, $cHCO_3^-$ coordinate system (Davenport diagram [7]). This was originally utilized by Van Slyke [8], and I therefore find it appropriate to call the equation for the pH, $cHCO_3^-$ equilibration curve the Van Slyke equation. *For plasma* the Van Slyke equation can be written:

$$\Delta cHCO_3^-(P) = -\beta H^+(X \subset P) \cdot \Delta pH(P) + \Delta cB'(P), \qquad (2)$$

where

$$\Delta cHCO_3^-(P) = \Delta cHCO_3^-(P^\circ|P) = cHCO_3^-(P) - 24.4 \text{ mmol/l},$$

$$\Delta pH\ (P) = \Delta pH(P^\circ|P) = pH(P) - 7.4,$$

$$\Delta cB'(P) = \Delta cB'(P^\circ|P) = cB'(P) - cB'(P^\circ).$$

P° is plasma titrated with strong acid or base to pH(P) = 7.40 at pCO_2 = 5.33 kPa and T = 310.15 K ($\Rightarrow cHCO_3^-$ = 24.4 mmol/l).

$\beta H^+(X \subset P)$ is the buffer value for H^+ due to non-bicarbonate buffers in the plasma. According to eq (20) of the preceding paper it can be expressed as:

$$\beta H^+(X \subset P) = \rho Pr(P) \cdot 0.11 \text{ mol/kg},$$

i.e., for normal plasma with $\rho Pr(P)$ = 70 g/l $\Rightarrow \beta H^+(X \subset P)$ = 7.7 mmol/l.

The Van Slyke equation is derived from:

$$\Delta cB'(P) = \Delta cHCO_3^-(P) + \Delta cX'(P),$$

and for pH(P) = 7.40 we have $\Delta cX'(P) = 0$ (compare eq (14) of the preceding paper).

For erythrocyte fluid the Van Slyke equation is analogous:

$$\Delta cHCO_3^-(E) = -\beta H^+(X \subset E) \times \Delta pH(E) + \Delta cB'(E), \qquad (3)$$

where E° is erythrocyte fluid in equilibrium with P°, *i.e.*, $pH(E^\circ)$ = 7.19 for pCO_2 = 5.33 kPa and 37 °C $\Rightarrow cHCO_3^-(E^\circ)$ = 12.6 mmol/l. The buffer value for H^+ due to the non-bicarbonate buffers is

$$\beta H^+(X \subset E) = 3.0 \cdot cHb(E),$$

i.e., for normal erythrocyte fluid with $c\mathrm{HB}(E) = 21$ mmol/l $\beta H^+(X \subset E) = 63$ mmol/l (see eq (21) of the preceding paper).

For whole blood the Van Slyke equation expressed in terms of the plasma bicarbonate concentration is:

$$\Delta c\mathrm{HCO}_3^-(P) = -B \cdot \Delta \mathrm{pH}(P) + \Delta c\mathrm{B}'(B)/Z \tag{4}$$

where $B = (\partial c\mathrm{HCO}_3^-(P)/\partial \mathrm{pH}(P))_{\Delta c\mathrm{B}'(B)}$ can be expressed by the following approximation (eq (41) of the preceding paper):

$$B = 2.3 \cdot c\mathrm{Hb}(B) + 7.7 \text{ mmol/l}.$$

Z is derived from eq (31) of the preceding paper:

$$\mathrm{pH}(P) = 7.40$$

$$\Delta c\mathrm{B}'(B) = \Delta c\mathrm{HCO}_3^-(B)$$

$$= \Delta c\mathrm{HCO}_3^-(P) \cdot (1 - (1 - rc) \cdot \phi\mathrm{E}(B)),$$

$$i.e. \quad Z = (1 - (1 - rc) \cdot \phi\mathrm{E}(B),$$

where $rc = c\mathrm{HCO}_3^-(E)/c\mathrm{HCO}_3^-(P) = 0.515$ (for $\mathrm{pH}(P) = 7.40$). For $c\mathrm{HB}(E) = 21$ mmol/l we therefore get:

$$Z = 1 - 0.023 \cdot c\mathrm{Hb}(B)/(\text{mmol/l}).$$

The relationship between the base excess concentration of plasma, erythrocyte fluid, and whole blood is the following:

$$\Delta c\mathrm{B}'(B) = \mathrm{P}(B) \cdot \Delta c\mathrm{B}'(P) + \phi\mathrm{E}(B) \cdot \Delta c\mathrm{B}'(E) \tag{5}$$

$$= (1 - \phi\mathrm{E}(B) \cdot \Delta c\mathrm{B}'(P) + \phi\mathrm{E}(B) \cdot \Delta c\mathrm{B}'(E).$$

$$\text{For } c\mathrm{Hb}(E) = 21 \text{ mmol/l} \Rightarrow \phi\mathrm{E}(B) = c\mathrm{HB}(B)/(21 \text{ mmol/l}).$$

3. pH(E) - pH(P) Equation

The relationship between pH(E) and pH(P) measured in normal blood can be expressed as follows (eq (27) of the preceding paper):

$$\mathrm{pH}(E) = 7.19 + 0.77 \cdot (\mathrm{pH}(P) - 7.40). \tag{6}$$

The coefficient 0.77 is due to a pH variation of the concentration of "non-diffusible" ions (*e.g.*, protein anions) in plasma and erythrocyte fluid. The coefficient can be calculated approximately from the buffer value for H^+ due to non-bicarbonate buffers in plasma and erythrocyte fluid, assuming the erythrocyte membrane is impermeable to cations for short term changes.

The value 7.19 is dependent on the membrane potential across the erythrocyte membrane (normally approximately -13.0 mV, inside negative) which is due to the active transport of Na^+ out of the erythrocytes. Inhibition of the Na^+ pump causes a diminution in the membrane potential and a swelling of the erythrocytes (hemolysis). Increased activity of the Na^+ pump causes an increase in the membrane potential and therefore a lower pH(E) for pH(P) = 7.40.

According to eq (38) of the preceding paper we have:

$$r_c = r_a \cdot 0.835,$$

where $r_c = c\mathrm{HCO}_3^-(\mathrm{E})/c\mathrm{HCO}_3^-(\mathrm{P})$, and $r_a = a\mathrm{HCO}_3^-(\mathrm{E})/a\mathrm{HCO}_3^-(\mathrm{P}) = a\mathrm{H}^+(\mathrm{P})/a\mathrm{H}^+(\mathrm{E})$. By combination of this equation and eq (6) we get:

$$c\mathrm{HCO}_3^-(\mathrm{E}) = c\mathrm{HCO}_3^-(\mathrm{P}) \cdot 0.835 \cdot \mathrm{antilog}\,(1.492 - 0.23\ \mathrm{pH(P)}) \qquad (7)$$

4. Comparison of the Van Slyke Equation and the Alignment Nomogram

The nomographic algorithm is based on a linear representation of the CO_2 equilibration curves of the blood in a pH, log pCO_2 coordinate system [3,4]. This algorithm is more difficult to express in a simple arithmetic form because the slope of the equilibration lines varies with both the hemoglobin concentration and the base excess concentration of the sample. In a pH, $c\mathrm{HCO}_3^-$ coordinate system the variation of the slope with the base excess concentration (or with pH) is small. Both algorithms are approximations. A comparison of the results obtained with the arithmetic algorithms and the nomographic algorithm (Alignment Nomogram) [4] is shown in table 1. The table includes values for the calculated

Table 1. The base excess concentration of the blood and the extracellular fluid calculated (A) by means of the alignment nomogram [4], (B) by means of the Van Slyke equation for whole blood (eq (4)), and (C) by means of the Van Slyke equations for plasma and erythrocyte fluid (eq (2) and (3)) together with the relationship between pH(P) and pH(E) (eq (6)). The calculations are based on a hemoglobin concentration of: $c\mathrm{Hb(b)}$ = 9.0 mmol/l, and $c\mathrm{Hb(Ecf)}$ = 3.6 mmol/l.

	Measured		Calculated	
	pH(P)	pCO_2(B) (mmHg)	$\Delta cB'$(B) (mmol/l)	$\Delta cB'$(Ecf) (mmol/l)
Acute hypercapnia	7.09	100	A -4.6	±0.0
			B -2.6	+0.5
			C -2.3	+1.0
Acute hypocapnia	7.70	16	A +0.5	-2.8
			B +2.9	-0.1
			C +3.0	-0.3
Chronic hypercapnia	7.30	80	A +8.2	+11.7
			B +9.2	+11.7
			C +9.4	+11.9
Chronic hypocapnia	7.44	25	A -5.2	-6.5
			B -5.2	-6.4
			C -5.1	-6.5
Acute base deficit	6.90	30	A -27.6	-25.0
			B -26.0	-24.4
			C -27.0	-24.3
(Chronic) base deficit	7.15	10	A -25.8	-24.6
			B -22.3	-22.9
			C -22.8	-22.9
(Chronic) base excess	7.60	55	A +26.0	+29.0
			B +27.4	+29.4
			C +26.7	+28.9

excess concentration of base in the average extracellular fluid (including blood). This value is calculated on the assumption that the buffer value for H^+ due to non-bicarbonate buffers in the extracellular fluid is mainly due to hemoglobin, and that the hemoglobin concentration of the total extracellular fluid (including blood) is approximately 3.6 mmol/l [4]. The advantage of this quantity is that it remains virtually constant during acute changes in the $pCO_2(B)$ *in vivo*, whereas the base excess concentration of blood and plasma varies in opposite directions due to a redistribution of H^+ between phases of different buffer value.

The results indicate that the base excess concentration of the blood or extracellular fluid can be calculated with sufficient accuracy for all clinical purposes by means of the arithmetic algorithms. In spite of the approximations made in the derivation of the Van Slyke equation for whole blood where the variation of B with $cHCO_3^-(P)$ at constant pH(P) is ignored, there seems to be no reason to use the more complicated algorithm based on the empirical relationship between pH(P) and pH(E). The major approximation undoubtedly lies in the use of a constant buffer value for non-bicarbonate buffers in plasma and erythrocyte fluid irrespective of a considerable pH variation.

When the pCO_2 value falls in the range between 25 and 80 mmHg, the alignment nomogram is likely to provide the best approximation, because it was constructed from experimental data in the pCO_2 range. When the pH is around 7.3 to 7.5 but the pCO_2 is very high or low, the Van Slyke equation is likely to provide the best approximation, because the non-bicarbonate buffer values is most accurate for this pH range.

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DETERMINATION OF TOTAL CO_2 CONCENTRATION IN BLOOD OR PLASMA

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Carbon dioxide is present in blood as dissolved CO_2, carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), carbonate (CO_3^{2-}) and carbamate, *i.e.*, CO_2 bound to free aminogroups of proteins ($RNHCOO^-$). The quantity total CO_2 concentration (c_{CO_2}) is defined as the sum of the concentrations of all forms in which CO_2 is present. In most methods [1][1] for the determination of c_{CO_2} in blood or plasma ($c^b_{CO_2}$ and $c^p_{CO_2}$, respectively), blood or plasma is added to an acid reagent for conversion of bound CO_2 (HCO_3^-, CO_3^{2-} and $RNHCOO^-$) into free CO_2 (H_2CO_3 and dissolved CO_2). As a measure for c_{CO_2} may then be used

1) the total amount of CO_2 which can be extracted from the mixture of sample and reagent (extraction methods);

2) the rise in P_{CO_2} in a given volume of gas which is equilibrated with the sample-reagent mixture (equilibration methods);

3) the rise in P_{CO_2} in the sample-reagent mixture when escape of any CO_2 is prevented.

In the method developed in our laboratory (the cediometer), the equilibration principle is applied [2,3].

1. Extraction Methods

Classic extraction methods are the volumetric [4] and the manometric Van Slyke techniques [5,6]. More recently gas chromatographic [7,8] or mass spectrometric [9] techniques have been applied to determine the total amount of CO_2 extracted from the sample-reagent mixture. The main problem in applying these techniques is to ascertain that all CO_2 has indeed been extracted from the sample-reagent mixture. Under favorable conditions, highly reproducible results can be obtained (coefficient of variation $\approx$1 percent).

The absolute accuracy depends on the reliability of the calibration procedure. When gas chromatographic or mass spectrometric techniques are used, Na_2CO_3 or $NaHCO_3$ reference

[1]Figures in brackets indicate the literature references at the end of this paper.

solutions are used for calibration. The accuracy then obviously depends on the reliability of the reference solutions.

The volumetric or manometric techniques are usually considered as absolute methods, *i.e.*, methods in which calibration with reference solutions is not required. The accuracy depends on the accuracy of the volume or pressure measurements. In the volumetric technique, CO_2 is extracted by shaking and vacuum exposure. The volume of the CO_2 set free is measured at barometric pressure. Corrections are necessary for other gases (O_2, N_2) set free from the sample-reagent mixture. Because of the uncertainty of these corrections the accuracy of the rather simple volumetric technique is dubious.

The more laborious manometric technique of Van Slyke and Neill [5] is usually considered as the most accurate method for determining c_{CO_2}. In this method, CO_2 is also extracted from the sample-reagent mixture by shaking and vacuum exposure. After all gas has been extracted, it is reduced to a known volume V and the pressure is determined. CO_2 is then absorbed using a concentrated NaOH solution and the pressure of the remaining gas is again measured at volume V. The pressure difference before and after absorption of CO_2 corresponds to the pressure in the volume V of the CO_2 set free from the sample. c_{CO_2} of the sample is calculated by multiplying the pressure difference with a factor depending on V, the sample volume, the acid reagent volume and the temperature. This factor, which can be obtained from reference [10], contains a correction for the amount of CO_2 redissolving in the sample-reagent mixture while the gas is reduced to volume V. The correction has been determined using Na_2CO_3 reference solutions. However, there is some doubt about the magnitude of this correction, because the amount of CO_2 that redissolves depends upon the apparatus used and upon the skill and speed of the operator. With utmost care and skill, highly reproducible c_{CO_2} determinations are possible (coefficient of variation $\approx$ 0.6 percent). However, because of the need for Na_2CO_3 reference solutions for finding the correction for CO_2 redissolving in the sample-reagent mixture, the manometric Van Slyke technique is no absolute method either.

2. Equilibration Methods

For the equilibration of a fixed gas volume with a fixed volume of blood or plasma after the addition to acid reagent, a closed system containing a circulating pump is usually employed. For the determination of the rise in P_{CO_2} of the gas mixture, photometric [11], potentiometric [2,3], gas chromatographic [12] and mass spectrometric techniques, as well as infrared gas analysis [13] can be used. Under favorable conditions highly reproducible results can be obtained (coefficient of variation $\approx$ 1 percent). Na_2CO_3 or $NaHCO_3$ reference solutions are used for calibration.

In the cediometer, equilibration of the sample-reagent mixture with a fixed gas volume is performed in a closed system consisting of a measuring chamber filled with $NaHCO_3$-NaCl solution, a sample chamber filled with acid reagent, and tubing connecting measuring chamber

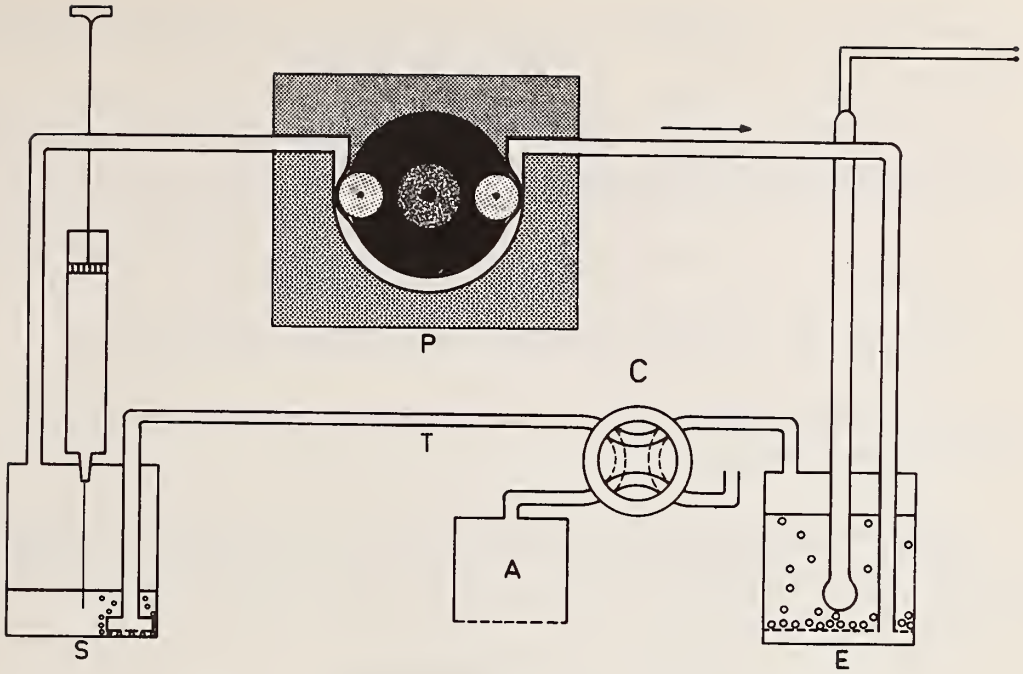

Figure 1. Schematic diagram of the closed gas-liquid circuit of the cediometer. E = measuring chamber; S = sample chamber; T = gas tubing; A = CO_2 absorber; C = cock; P = pump.

and sample chamber (fig. 1). The gas mixture is driven through the system by means of a pump. The rise in P_{CO_2} in the gas mixture caused by adding a constant volume of blood or plasma to the acid reagent, is determined by the simultaneous equilibration of the gas mixture with the HCO_3^--NaCl solution and measuring the ensuing change of pH (ΔpH_e) by means of a combined glass-Ag/AgCl electrode. ΔpH_e is a measure of the total CO_2 concentration of the sample injected into the acid reagent. The method is easy to perform; the time necessary for a determination is about 3 minutes. The coefficient of variation is 0.8 percent.

3. Methods Based on Rise in P_{CO_2} of Sample-Reagent Mixture

For the determination of the rise in P_{CO_2} of the sample-reagent mixture, a CO_2 electrode can be used. The method is quite simple and rather reproducible results have been reported, the coefficient of variation being ≈ 1.2 percent [14]. Na_2CO_3 or $NaHCO_3$ reference solutions are used for calibration.

4. Discussion

In spite of the simplicity of many available methods for determining c_{CO_2}, common practice in most laboratories is to measure pH and P_{CO_2} and to calculate c_{CO_2} or $c_{HCO_3^-}$ using the Henderson-Hasselbalch equation. Yet the direct determination of c_{CO_2} has some distinct advantages over that of P_{CO_2}.

1) *Calibration* can easily be performed with Na_2CO_3 or $NaHCO_3$ reference solutions, which can be prepared from commercially available analytical grade reagents.

2) In contrast to P_{CO_2}, c_{CO_2} is *independent of temperature*. Therefore, mistakes due to incorrect thermostating of the measuring system are excluded.

3) c_{CO_2} is *far less influenced by metabolism* of anaerobically stored blood than P_{CO_2}.

4) c_{CO_2} is *less influenced by escape of* CO_2 from the sample than P_{CO_2}.

5) The instrumentation required for measuring c_{CO_2} is *less vulnerable* than membrane covered CO_2 electrodes are.

Because of these advantages, the determination of c_{CO_2} should be recommended, especially in smaller laboratories where some time passes between sampling and analysis or between the determination of pH and P_{CO_2}.

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THE APPARENT OVERALL FIRST DISSOCIATION CONSTANT OF CO_2 IN PLASMA

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Since Hasselbalch [1][1] in 1916 put the equilibrium equation for the dissociation of carbonic acid in the logarithmic form, many investigations have been devoted to the determination of the apparent *overall* first dissociation constant of CO_2 in plasma (table 1).

$$pH = pK_1' + \log \frac{c_{HCO_3^-}}{S \cdot P_{CO_2}} \qquad (1)$$

pK_1' denotes the negative logarithm of the apparent *overall* first dissociation constant of CO_2, $c_{HCO_3^-}$ the concentration of HCO_3^- ($mmol \cdot l^{-1}$), S the solubility coefficient[2] of CO_2 ($mmol \cdot l^{-1} \cdot (mm\ Hg)^{-1}$) and P_{CO_2} the CO_2 tension (mm Hg). Introduction of any new method for the determination of pH, $c_{HCO_3^-}$ or P_{CO_2} invariably resulted in redetermination of pK_1'. It proved that the value of pK_1' depended on the analytical methods. To stress the experimental nature of the constant, the symbol $p\hat{K}_1'$ was introduced, denoting the value calculated for pK_1' using eq. (1) when experimentally found values for pH, $c_{HCO_3^-}$, P_{CO_2} and S are used. In the $p\hat{K}_1'$ determinations of the twenties and early thirties [2-7], P_{CO_2} was calculated from F_{CO_2} of the gas mixture with which the plasma had been equilibrated. pH was measured using hydrogen electrodes. $c_{HCO_3^-}$ was calculated from the total CO_2 concentration c_{CO_2} as measured with the manometric Van Slyke technique, assuming c_{CO_2} to be equal to the sum of $c_{HCO_3^-}$ and $S \cdot P_{CO_2} \cdot S$ had been determined by experiments in which acidified plasma or serum was equilibrated with gas mixtures of known P_{CO_2} and c_{CO_2} measured manometrically. A mean value of 6.10 was found for $p\hat{K}_1'$ at body temperature with no appreciable variation, even when abnormal plasma was used.

However, with the introduction of newer methods for pH and P_{CO_2} measurement, doubt arose about the constancy of $p\hat{K}_1'$. The introduction of glass electrodes for the determination of pH resulted in reports in which a decrease of the value of $p\tilde{K}_1'$ with increasing pH was stated, $\Delta p\tilde{K}_1'/\Delta pH$ ranging from -0.03 to -0.06 [8-10,12]. The introduction of CO_2 electrodes for the direct measurement of P_{CO_2} resulted in investigations [13,14] in which appreciable variations of $p\tilde{K}_1'$ in seriously ill patients were measured (from 5.96 to 6.27). The tem-

[1]Figures in brackets indicate literature references at the end of this paper.

[2]$S = (c_{CO_2\ \text{dissolved}} + c_{H_2CO_3}) \cdot (P_{CO_2})^{-1}$.

Table 1. Results of previous $p\mathring{K}_1'$ determinations.

Authors	Material	Temp °C	pH range	n[a]	$p\mathring{K}_1'$[b] (pH = 7.4)	Range	$\Delta p\mathring{K}_1'/\Delta t$(°C)	$\Delta p\mathring{K}_1/\Delta t$(°C) (pH = 7.4)	$p\mathring{K}_1'$[b] (t = 37°) (pH = 7.4)
Warburg [2]	Horse and ox serum	38	6.43 -8.01	23	6.115	6.074-6.144	-0.0055		6.120
		20	6.31 -8.03	25	6.214	6.160-6.255			
Cullen [3]	Horse serum and plasma	38	7.32 -7.64	15	6.108	6.082-6.131			6.111
Van Slyke *et al.* [4]	Horse serum	38	7.06 -7.64	13	6.126	6.086-6.170			6.129
Cullen *et al.* [5]	Dog serum and pathological human serum	38	7.214-7.507	18	6.069	6.044-6.096	-0.0040		6.073
		20	7.357-7.505	8	6.141	6.121-6.167			
Hastings *et al.* [6]	Normal and pathological human serum	38	7.225-7.553	16	6.105	6.094-6.124			6.108
Robinson *et al.* [7]	Normal and pathological dog serum, pathological human serum	38	7.057-7.561	225	6.090	6.071-6.108			6.093
Dill *et al.* [8]	Human, dog and ox serum	37	6.7 -8.1	18	6.112	6.07 -6.1[d]		-0.030	6.112
Severinghaus *et al.* [9]	Human and dog plasma	37.5	6.7 -8.0	26	6.086	6.05 -6.14	-0.0048[c]	-0.044	6.088
		24	6.7 -8.0	13	6.151	6.12 -6.20	-0.0063[d]	-0.063	
Siggaard-Anderson [10]	Human plasma (a)	38	7.0 -7.6	16	6.099	6.08 -6.12	-0.0033[e]	-0.047	6.102
		25	7.0 -7.6	16	6.142	6.12 -6.18	-0.0039[f]	-0.055	
	(b)	38	7.30 -7.45	4	6.108	6.103-6.111			6.111
Albers *et al.* [11]	Dog plasma	38		12	6.088	SD = 0.014			6.091
Maas [12]	Human plasma and serum	38	6.4 -7.8	60	6.101	6.07 -6.13	-0.0032	-0.040	6.104
		25	6.2 -7.8	42	6.142	6.12 -6.16			

[a] n = number of determinations.

[b] $p\mathring{K}_1'$ recalculated using $\mathcal{S}'$ values as given by Austin *et al.*[1].

[c] pH = 7.4.

[d] pH = 6.8.

[e] pH = 7.4.

[f] pH = 7.0.

perature dependence $\Delta p\tilde{K}_1'/\Delta T$, usually inferred from measurements of but two temperatures, was reported to vary between -0.0032 and -0.0063 $p\tilde{K}_1'$ unit·(°C)$^{-1}$.

After having developed a new method for the determination of c_{CO_2} in blood or plasma [15] and a tonometry set-up [16] allowing blood or plasma to be equilibrated with gas mixtures of known composition, we decided to redetermine $p\tilde{K}_1'$ over the temperature range of 16-42 °C and the pH range of 6.8-7.8. Plasma of healthy volunteers was equilibrated in horizontally rotating tonometers at 16, 20, 26, 30, 32.5, 35, 37.5 and 42.5 °C with CO_2/O_2 gas mixtures from cylinders, F_{CO_2} ranging from 2-16 percent. F_{CO_2} was measured with a Haldane gas analysis apparatus, the standard deviation calculated from duplicate determinations being 0.04 percent. After equilibrium, two 5 ml samples were taken from each tonometer. Plasma pH was measured with capillary microelectrodes (Radiometer E5021a) connected to a direct reading pH meter (Radiometer PHM27) or a balancing pH meter (Radiometer PHM4). Level and slope of the pH measuring system were controlled with two NBS buffers, the slope daily, the level before and after each pH determination. In each sample pH was measured in duplicate; the standard deviation was 0.006 pH unit. Plasma c_{CO_2} was measured in each sample in duplicate using a cediometer; the coefficient of variation was 0.8 percent. $p\tilde{K}_1'$ was calculated as

$$p\tilde{K}_1' = pH - \log \frac{c_{CO_2} - S \cdot P_{CO_2}}{S \cdot P_{CO_2}} \quad . \tag{2}$$

For S the values given by Austin *et al.* [17] were used.

A total of 555 duplicate determinations of $p\hat{K}_1'$ in plasma equilibrated at known temperature with gas mixtures of known F_{CO_2} were performed (table 2). The standard deviation as calculated from the duplicate determinations was 0.008. The best approximation of the relationship between $p\hat{K}_1'$, T and pH was found to be [18].

$$p\hat{K}_1' = -4.7416 + \frac{1840.141}{T} + 0.015906\ T - \log\left(1 + \frac{0.020682}{10^{-pH+7}}\right) . \tag{3}$$

The mean difference between the experimental $p\hat{K}_1'$ values and those from eq. (3) was 0.0000 ± 0.0138 (SD). Figure 1 shows a curve relating $p\hat{K}_1'$ to temperature as calculated with eq. (3) at pH = 7.4. The curve is in good agreement with the experimental data presented, as well as with those reported by other investigators. Figure 1 also demonstrates that the estimation of the relationship between $p\hat{K}_1'$ and temperature on the basis of $p\hat{K}_1'$ determinations at but two temperatures is subject to substantial error, as $p\hat{K}_1'$ does not vary linearly with temperature.

The decrease of $p\hat{K}_1'$ with increasing pH reported by other investigators has been confirmed in the present investigation and is represented by the term $-\log(1 + 0.020682/10^{-pH+7})$ in eq. (3). The decrease of $p\hat{K}_1'$ with increasing pH has been ascribed to a pH dependent error in the determination of $c_{HCO_3^-}$ [10], to an alkaline error of glass electrodes [9] or to both.

Table 2. Experimental and calculated $p\hat{K}_1'$ at different temperatures and pH.

Temp. °C	pH range	pH mean	$p\hat{K}_1'$ (exp.) mean	$p\hat{K}_1'$ (calc.)	Difference	SD of difference
42.5	6.8-7.3	7.167	6.091	6.096	-0.005	0.0068
(n = 23)	7.3-7.5	7.362	6.097	6.089	+0.008	0.0105
	7.5-7.8	7.542	6.083	6.079	+0.004	0.0030
37.5	6.8-7.3	7.181	6.113	6.110	+0.003	0.0124
(n = 136)	7.3-7.5	7.422	6.101	6.100	+0.001	0.0142
	7.5-7.8	7.603	6.090	6.088	+0.002	0.0178
35.0	6.8-7.3	7.162	6.110	6.119	-0.009	0.0102
(n = 33)	7.3-7.5	7.455	6.103	6.107	-0.004	0.0115
	7.5-7.8	7.599	6.087	6.097	-0.010	0.0153
32.5	6.8-7.3	7.191	6.119	6.127	-0.008	0.0166
(n = 35)	7.3-7.5	7.457	6.113	6.116	-0.003	0.0069
	7.5-7.8	7.633	6.103	6.104	-0.001	0.0122
30.0	6.8-7.3	7.149	6.142	6.138	+0.004	0.0131
(n = 74)	7.3-7.5	7.415	6.129	6.128	+0.001	0.0134
	7.5-7.8	7.661	6.118	6.111	+0.007	0.0156
26.0	6.8-7.3	7.131	6.156	6.156	-	0.0114
(n = 58)	7.3-7.5	7.422	6.141	6.145	-0.004	0.0098
	7.5-7.8	7.622	6.128	6.132	-0.004	0.0112
20.0	6.8-7.3	7.120	6.191	6.188	+0.003	0.0144
(n = 92)	7.3-7.5	7.387	6.179	6.178	+0.001	0.0126
	7.5-7.8	7.616	6.162	6.164	-0.002	0.0127
16.0	6.8-7.3	7.050	6.212	6.212	-	0.0167
(n = 85)	7.3-7.5	7.382	6.202	6.200	+0.002	0.0139
	7.5-7.8	7.561	6.185	6.190	-0.005	0.0164

Obviously, as $c_{HCO_3^-}$ is calculated as ($c_{CO_2} - S \cdot P_{CO_2}$) or determined by titration, CO_3^{2-} and $RNHCOO^-$ present in plasma are included. The value used for $c_{HCO_3^-}$ in calculating $p\hat{K}_1'$ will be too high and $p\hat{K}_1'$ consequently lower than pK_1'. As the fraction of bound CO_2 present as CO_3^{2-} or as $RNHCOO^-$ increases with increasing pH, the error increases with increasing pH. However, $c_{CO_3^{2-}}$ and c_{RNHCOO^-} are too small even at higher pH to completely explain the observed decrease of $p\hat{K}_1'$ with increasing pH. An alkaline error of the glass electrode might in part be responsible.

The scatter of the $p\tilde{K}_1'$ values of table 1 and of the present investigation is much smaller than that reported for $p\tilde{K}_1'$ in disease when measuring P_{CO_2} and pH in whole blood, using membrane covered electrodes for the determination of P_{CO_2}. Some changes of $p\tilde{K}_1'$ (and thus of $p\tilde{K}_1'$) may be expected to result from changes in the ionic strength of plasma. However, the changes of the ionic strength of plasma in disease are too small to be responsible. Because of the unequal distribution of dissolved CO_2 and HCO_3^- over the water and non-water phase of plasma, the value of $p\tilde{K}_1'$ found in plasma differs slightly from that in simple electrolyte solutions of the same ionic strength. Changes of the distribution ratio of CO_2 and HCO_3^- during disease might cause a change of $p\tilde{K}_1'$. However, these changes have been calculated to be small [19]. Variations of $p\tilde{K}_1'$ in disease must therefore largely be ascribed to unknown errors in the determination of pH, P_{CO_2} or $c^p_{HCO_3^-}$ in abnormal plasma.

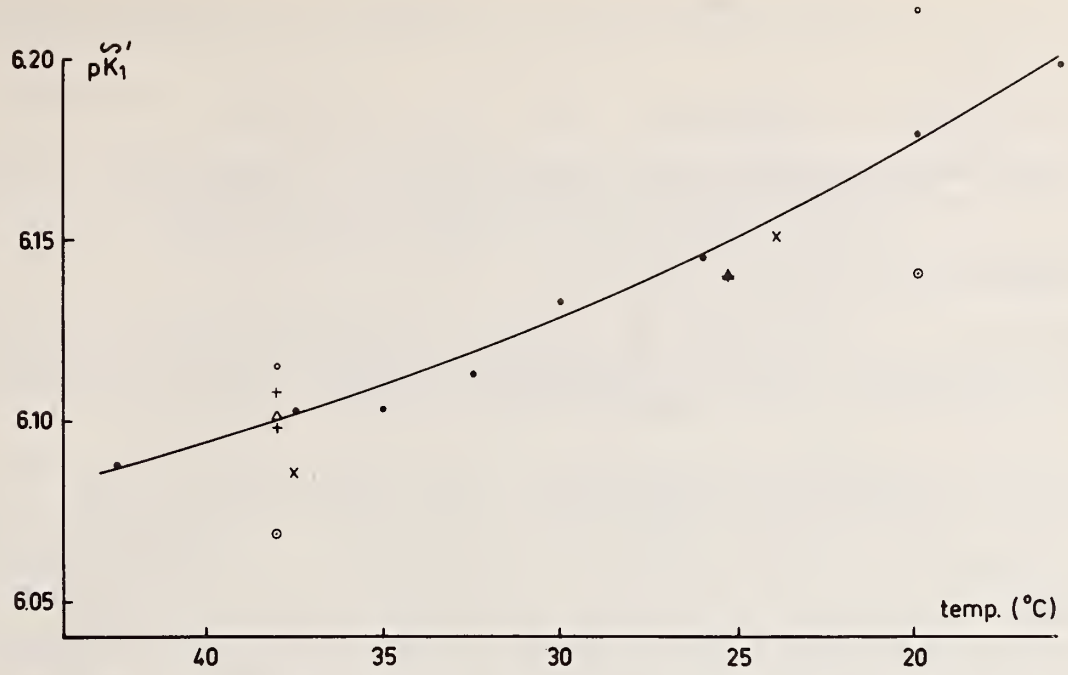

Figure 1. Relationship between the experimentally determined apparent first dissociation constant of carbonic acid in human plasma and temperature. The curve represents eq. (3) at $pH_p = 7.4$

•	present investigation	o	Warburg [2]
⊙	Cullen *et al.* [5]	x	Severinghaus *et al.* [9]
+	Siggaard-Andersen [10]	Δ	Maas [12]

The $p\tilde{K}_1'$ values taken from the literature have been recalculated, using for S' the values reported by Austin *et al.* [17].

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QUANTITATIVE RELATIONSHIPS BETWEEN TOTAL CO_2 CONCENTRATION IN BLOOD AND PLASMA, PLASMA BICARBONATE CONCENTRATION, PLASMA pH AND CARBON DIOXIDE TENSION BETWEEN 16-42 °C

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In our laboratory the acid-base status of patients or experimental animals is evaluated by determining total CO_2 concentration in blood ($c^b_{CO_2}$), blood pH at 37 °C ($pH_b(37)$), haemoglobin concentration (c_{Hb}) and oxygen saturation (S_{O_2}). From these quantities plasma pH at 37 °C ($pH_p(37)$), total CO_2 concentration in plasma ($c^p_{CO_2}$), plasma bicarbonate concentration ($c^p_{HCO_3^-}$) and blood pH and carbon dioxide tension at body temperature ($pH_b(T_b)$ and $P_{CO_2}(T_b)$) are calculated [1][1].

$pH_p(37)$ is calculated from $pH_b(37)$ using eq. (1)

$$pH_p = pH_b + 0.01 \ . \tag{1}$$

$c^p_{CO_2}$ and $c^p_{HCO_3^-}$ are practically independent of temperature in anaerobically stored blood and can be calculated from the measured quantities without considering body temperature T_b. $c^p_{CO_2}$ is calculated from $c^b_{CO_2}$ using eq. (2)

$$c^p_{CO_2} = c^b_{CO_2} \frac{\left(2.244 - 0.422\, S_{O_2}\right) \cdot \left(8.74 - pH_b(37)\right)}{\left(2.244 - 0.422\, S_{O_2}\right) \cdot \left(8.74 - pH_b(37)\right) - 0.0288 c_{Hb}} \ . \tag{2}$$

$c^p_{HCO_3^-}$ can be calculated from $c^p_{CO_2}$ and $pH_p(37)$ using the Henderson-Hasselbalch equation, which for this purpose may be written as

$$c^p_{HCO_3^-} = \frac{c^p_{CO_2}}{1 + 10^{p\hat{K}'_1 - pH_p(37)}} \tag{3}$$

The value to be used for $p\hat{K}'_1$ at 37 °C and $pH_p(37)$ is calculated using eq. (4)

$$p\hat{K}'_1 = -4.7416 + \frac{1840.141}{T} + 0.015906\, T - \log\left(1 + \frac{0.020682}{10^{-pH_p(T)} + 7}\right) (T \text{ in } °K). \tag{4}$$

[1]Figures in brackets indicate the literature references at the end of this paper.

In contrast to $c^p_{CO_2}$ and $c^p_{HCO_3^-}$, pH_b and P_{CO_2} strongly depend upon temperature. $pH_b(T_b)$ is calculated using eq. (5)

$$pH_b(T_b) = pH_b(37) + (37 - T_b) \cdot \left(0.0151 + 0.0058\ (pH_p(37) - 7.4)\right) \quad (T_b \text{ in } °C). \tag{5}$$

$P_{CO_2}(T_b)$ is calculated from $pH_p(T_b)$ and $c^p_{CO_2}$ using the Henderson-Hasselbalch equation, which for this purpose is written as

$$P_{CO_2}(T_b) = \frac{c^p_{CO_2}}{S \cdot \left(10^{pH_p(T_b) - pK'_1} + 1\right)} \quad . \tag{6}$$

The value to be used for S at T_b is calculated from eq. (7), which has been derived from data of Austin *et al.* [2]

$$S = -3.24615 + \frac{536.734}{T} + 0.0049854\ T \quad (T \text{ in } °K) \quad . \tag{7}$$

The value to be used for $pH_p(T_b)$ is calculated from $pH_b(T_b)$ using eq. (1), the value to be used for pK'_1 at T_b and $pH_p(T_b)$ is calculated using eq. (4).

Using the eqs. (1) through (7), a computer program can be made for the rapid calculation of $c^p_{HCO_3^-}$ and $pH_b(T_b)$ and $P_{CO_2}(T_b)$ at any temperature between 16 and 42 °C. When no computer is available, the nomogram presented in figure 1 may be used [3]. This nomogram is an extension of the nomogram described by Brunsting [4] which is, in turn, a modification of the Singer-Hastings nomogram [5]. The nomogram is valid for oxygenated blood only. Correction for desaturation is possible with the aid of the data of table 1.

Obviously, eqs. (1) through (7) can also be used to calculate $pH_b(T_b)$, $P_{CO_2}(T_b)$, $c^p_{HCO_3^-}$, $c^p_{CO_2}$ and $c^p_{CO_2}$ when $pH_b(37)$, $P_{CO_2}(37)$, c_{Hb} and S_{O_2} have been measured or to calculate $pH_b(37)$, $pH_b(T_b)$, $P_{CO_2}(T_b)$, $c^p_{HCO_3^-}$ and $c^p_{CO_2}$, when $c^b_{CO_2}$, $P_{CO_2}(37)$, c_{Hb} and S_{O_2} have been measured.

1. Calculation of pH_p.

It has been found that there is a slight difference between pH_b and pH_p [6]. This difference is probably due to the effect of haemolysis at the interface between saturated KCl and blood on the liquid junction potential during the measurement of blood pH [7-9].

2. Calculation of $c^p_{CO_2}$.

Within the pH range occurring in blood *in vivo*, c_{CO_2} in red cells ($c^c_{CO_2}$) is smaller than $c^p_{CO_2}$. The difference is mainly due to the impermeability of the red cell membrane for haemoglobin, organic phosphate and plasma protein ions, causing a Gibbs-Donnan distribution

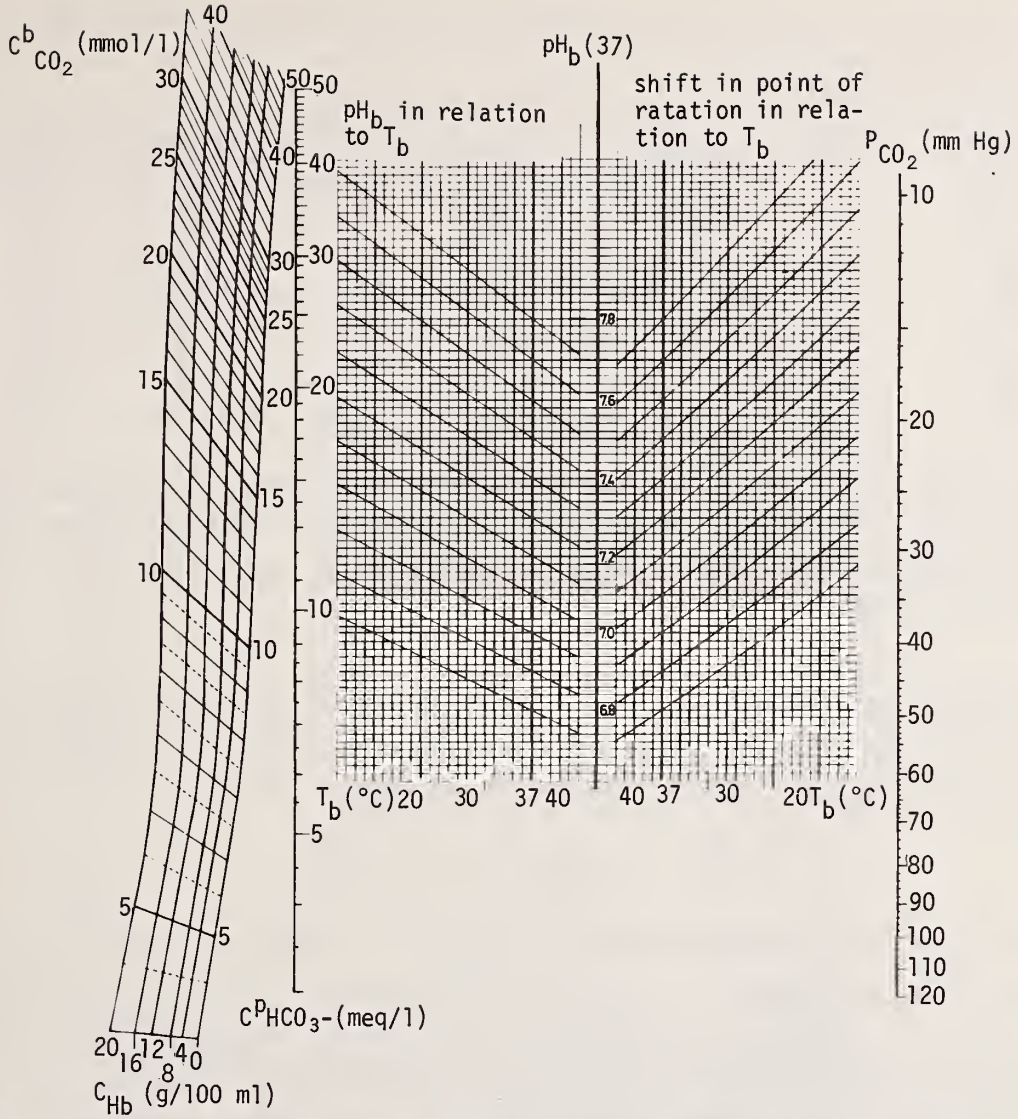

Figure 1. Nomogram to derive $c^p_{CO_2}$, $c^p_{HCO_3^-}$, P_{CO_2} at body temperature ($P_{CO_2}(T_b)$) and pH_b at body temperature ($pH_b(T_b)$) from pH_b at 37 °C ($pH_b(37)$), $c^b_{CO_2}$, c_{Hb} and T_b. To derive $c^p_{CO_2}$ and $c^p_{HCO_3^-}$ a line is drawn through the points corresponding to the measured values of pH_b (37), $c^b_{CO_2}$ and c_{Hb}. $c^p_{CO_2}$ is then read at the point where the $c^b_{CO_2}$ scale for c_{Hb} = 0 is intersected, and $c^p_{HCO_3^-}$ at the point where the $c^p_{HCO_3^-}$ scale is intersected. P_{CO_2} at 37 °C ($P_{CO_2}(37)$) is read at the point where the P_{CO_2} scale is intersected. To find P_{CO_2} (T_b) when T_b differs from 37 °C, a line has to be drawn through the point corresponding to the value found for $c^p_{CO_2}$ (at the $c^b_{CO_2}$ scale for c_{Hb} = 0) and another point at the central H_b (37) scale. The latter point may be found using the lines representing the shift of this point with temperature for a given value of H_b (37) in the right hand grid. In the right hand grid the line through the measured value of pH_b(37). is followed up (or down) to the point of intersection with the line corresponding to the actual value of T_b. The point of intersection is then projected on the central pH_b(37) scale. The lines of the left hand grid represent the change of pH_b with temperature for a given value of pH_b(37).

Table 1. Correction factors for $c^p_{HCO_2^-}$ and P_{CO_2} for deoxygenated blood.

c_{Hb} g/l	$pH_b(37)$ 6.8	7.0	7.2	7.4	7.6	7.8
20	0.3	0.3	0.4	0.5	0.5	0.7
40	0.6	0.7	0.8	0.9	1.1	1.3
60	1.0	1.1	1.2	1.4	1.7	2.1
80	1.3	1.5	1.7	1.9	2.3	2.8
100	1.6	1.8	2.1	2.5	2.9	3.7
120	2.0	2.2	2.6	3.0	3.6	4.5
140	2.3	2.7	3.1	3.6	4.3	5.5
160	2.7	3.1	3.6	4.2	5.1	6.5
180	3.1	3.5	4.1	4.8	5.9	7.5
200	3.5	4.0	4.6	5.5	6.7	8.7

The figures given represent the percentage to be subtracted from the value read for $c^p_{HCO_2^-}$ and for P_{CO_2} in the nomogram of figure 1, when the blood is completely deoxygenated. When the blood is partly oxygenated the correction factor is found by multiplication of the figures given with $(1 - S_{O_2})$.

of the diffusible ions, amongst them HCO_3^-. At a given haemoglobin concentration within the red cells (MCHC = c^c_{Hb}) the Gibbs-Donnan ratio mainly depends upon pH_b and S_{O_2} [10], whereas at given $c^p_{CO_2}$ and $c^c_{CO_2}$, $c^b_{CO_2}$ depends upon the haematocrit H,

$$c^b_{CO_2} = H \cdot c^c_{CO_2} + (1 - H) \cdot c^p_{CO_2} \qquad (8)$$

Thus, the ratio $c^p_{CO_2}/c^b_{CO_2}$ depends both upon H and c^c_{Hb} and furthermore upon pH_b and S_{O_2}. Therefore, it seems preferable to base empirical equations for calculating $c^p_{CO_2}$ from $c^b_{CO_2}$ upon c_{Hb} (= $c^c_{Hb} \cdot H$), pH_b and S_{O_2}. Equation (2) was derived by McHardy [11] from an equation given by Visser [12] who derived his equation from a nomogram given by Van Slyke and Sendroy [13]. The validity of eq. (2) was tested [9] using 317 blood samples in which $c^b_{CO_2}$, $c^p_{CO_2}$, $pH_b(37)$, c_{Hb} and S_{O_2} were measured. Of the 317 blood samples, 83 were centrifuged at 37 °C, the remaining 234 at room temperature. Results are shown in table 2. The mean difference between $c^p_{CO_2}/c^b_{CO_2}$ calculated using eq. (2) and $c^p_{CO_2}/c^b_{CO_2}$ measured directly is but small. The

Table 2. Difference between $c^p_{CO_2}/c^b_{CO_2}$ derived from $pH_b(37)$, S_{O_2} and c_{Hb} (or H or O_2 capacity), and $c^p_{CO_2}/c^b_{CO_2}$ calculated directly.

Centrifuged at (°C)	n[a]	Mean difference	SD[b]	Remarks
37	83	+0.007	0.027	equation (2)
19-25	234	+0.014	0.034	equation (2)
19-25 and 37	317	+0.012	0.032	equation (2)

[a] number of experiments
[b] standard deviation

mean difference between calculated and measured values of $c^p_{CO_2}/c^b_{CO_2}$ of blood samples centrifuged at room temperature is slightly greater than that found for samples centrifuged at 37 °C. However, eq. (2) may be used to calculate $c^p_{CO_2}$ from $c^b_{CO_2}$ at lower T_b as well as at T_b = 37 °C without introducing an appreciable systematic error. Figure 2 illustrates that eq. (2) is valid over the entire $c^p_{CO_2}$ range encountered in blood *in vivo*.

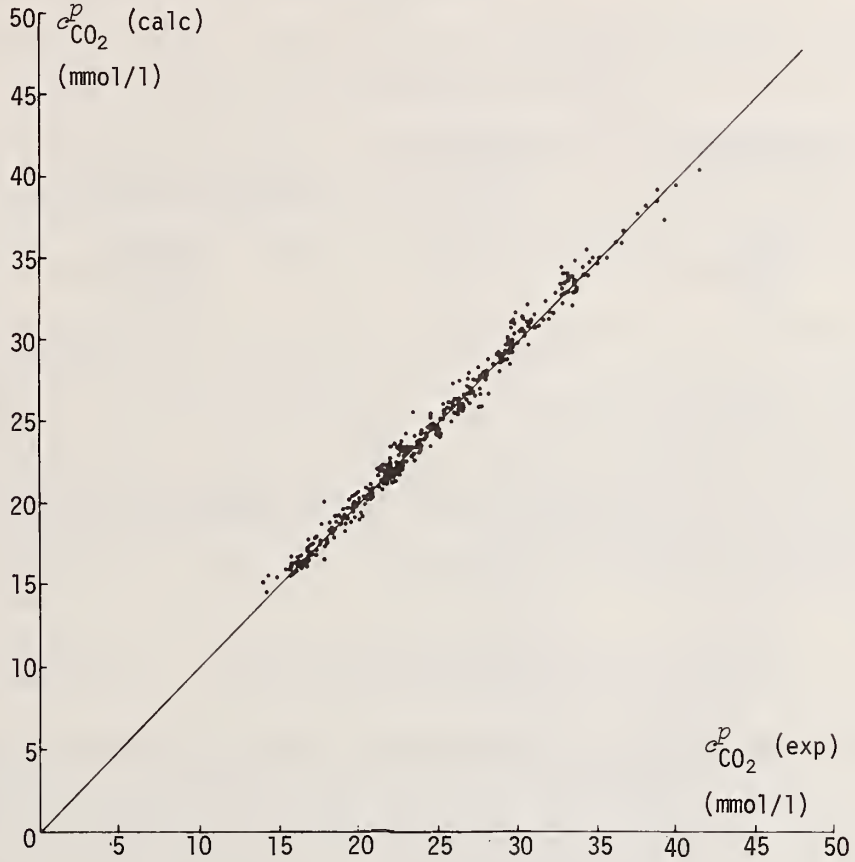

Figure 2. Relationship between $c^p_{CO_2}$ calculated from $c^b_{CO_2}$, $pH_b(37)$, c_{Hb} and S_{O_2} with eq. (2) $\left(c^p_{CO_2}(\text{calc})\right)$ and $c^p_{CO_2}$ measured directly $\left(c^p_{CO_2}\ (\text{exp})\right)$.

3. Calculation of $c^p_{HCO_3^-}$

In contrast to eq. (6) (*v.i.*) an overall check of the validity of eq. (3) is not possible, because $c^p_{HCO_3^-}$ cannot be measured independently of $c^p_{CO_2}$ or pH_p. However, because the denominator of the quotient in eq. (3) differs but slightly from unity (in the pH range 7.8-7.0, it ranges from 1.020 to 1.126), the inaccuracy in pK'_1 and pH_p has a negligible effect on $c^p_{HCO_3^-}$ and the accuracy with which $c^p_{HCO_3^-}$ is determined depends upon that of $c^p_{CO_2}$ only.

4. Calculation of $pH_b(T_b)$.

pH_b of anaerobically stored blood increases with decreasing temperature, because the acid reaction of both carbonic acid, haemoglobin and plasma decreases at decreasing temper-

ature. Theoretically [9], $\Delta pH_b/\Delta T$ depends upon $pH_b(37)$, $c^b_{CO_2}$ and $c_{Hb} \cdot S_{O_2}$ has only a minor influence. Equation (5) was derived from experiments with bovine blood, in which the influence of $c^b_{CO_2}$ and c_{Hb} proved negligible. The validity of eq. (5) for human and canine blood was checked by measuring $pH_b(37)$ and $pH_b(30)$ of 336 human and 829 canine blood samples. In addition, $pH_b(37)$ and $pH_b(20)$ was measured in 52 human blood samples and $pH_b(37)$ and $pH_b(16)$ in 68 human blood samples. Results are shown in table 3. An excellent agreement between

Table 3. Mean difference and standard deviation between $pH_b(30)$, $pH_b(20)$ and $pH_b(16)$ calculated from $pH_b(37)$ and $pH_b(30)$, $pH_b(20)$ and $pH_b(16)$ measured directly.

Blood	Number of measurements	Temperature (°C)	pH_b(meas)-pH_b(calc)	SD
canine	829	30	+0.001	0.016
human	336	30	-0.003	0.015
human	58	20	-0.031	0.026
human	68	16	-0.040	0.015

calculated and measured $pH_b(30)$ was found both in human and canine blood. At 20 °C and 16 °C the calculated values are somewhat lower than the measured ones. No explanation for this discrepancy can be given. It is in contrast with the good agreement of P_{CO_2} at 16 °C calculated from $pH_b(37)$, $c^b_{CO_2}$, c_{Hb} and S_{O_2} with P_{CO_2} in the gas mixture with which the blood had been equilibrated at 16 °C (*v.i.*).

5. Calculation of $P_{CO_2}(T_b)$.

It is clear from eq. (6), that the accuracy with which $P_{CO_2}(T_b)$ can be calculated from $pH_b(37)$, $c^b_{CO_2}$, c_{Hb} and S_{O_2}, depends upon the reliability with which $c^p_{CO_2}$ is calculated from $c^b_{CO_2}$ (eq. (2)) and $pH_p(T_b)$ is calculated from $pH_b(37)$ (eqs. (1) and (5)), as well as on the validity of eqs. (4) and (7) for calculating pK'_1 and S. The overall accuracy of the determination of $P_{CO_2}(T_b)$ was evaluated by equilibrating human blood with $CO_2/O_2/N_2$ mixtures of known

Table 4. Mean difference and standard deviation of P_{CO_2} calculated at tonometer temperature from $pH_b(37)$, $c^b_{CO_2}$, c_{Hb} and S_{O_2}, and P_{CO_2} of tonometry gas mixture.

P_{CO_2} range (mm Hg)	37 °C	30 °C	16 °C
10-30	+1.2 ± 0.9 n = 57	+0.6 ± 1.2 n = 38	+0.6 ± 1.1 n = 15
30-50	+0.8 ± 1.7 n = 48	+0.1 ± 2.6 n = 17	+0.8 ± 2.3 n = 15
50-80	-0.3 ± 2.0 n = 61	-0.9 ± 2.3 n = 36	+0.6 ± 4.3 n = 14
80-120	-1.9 ± 3.7 n = 27	-4.4 ± 4.4 n = 13	

composition, measuring $c^{b}_{CO_2}$, $pH_b(37)$, c_{Hb} and S_{O_2}, calculating $P_{CO_2}(T_b)$ from the measured values and comparing the calculated $P_{CO_2}(T_b)$ with P_{CO_2} in the gas mixture used. One hundred ninety three blood samples were equilibrated at 37 °C, 104 at 30 °C and 44 at 16 °C. Results are shown in table 4. At P_{CO_2} = 10-80 mm Hg a fair agreement is found. The lower values for blood with P_{CO_2} > 80 mm Hg, most evident in samples equilibrated at 30 °C, may be due to loss of CO_2 in the sampling or analysis procedure.

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P_{CO_2} INDEPENDENT QUANTITIES, WHY OR WHY NOT

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In our laboratories, disturbances of acid-base balance are assessed by plotting plasma pH at 37 °C ($pH_p(37)$) against plasma bicarbonate concentration ($c^p_{HCO_3^-}$) in a pH_p-$c^p_{HCO_3^-}$ diagram (fig. 1). Such a diagram, first introduced by Van Slyke [1][1] and popularized by

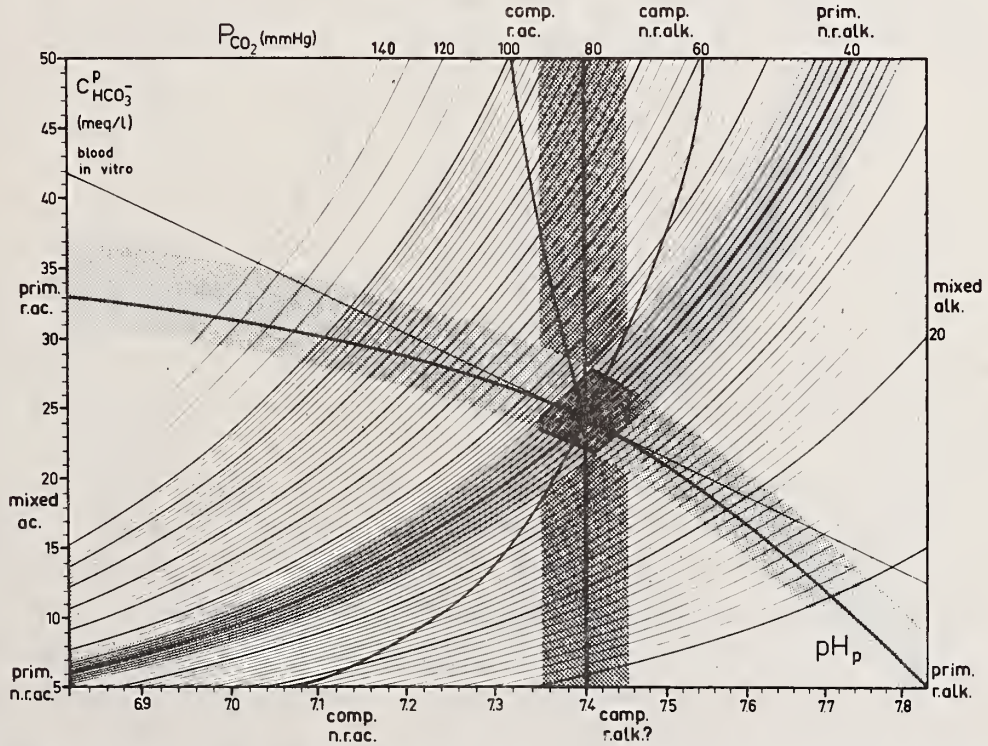

Figure 1. A $pH_p(37)$-$c^p_{HCO_3^-}$ diagram with normal area and the area in which pH_p and $c^p_{HCO_3^-}$ may be expected to fall during primary and compensated respiratory and non-respiratory disturbances and during mixed disturbances of acid-base balance; r.ac. = respiratory acidosis; r.alk. = respiratory alkalosis; n.r.ac. = non-respiratory acidosis; n.r.alk. = non-respiratory alkalosis.

[1] Figures in brackets indicate literature references at the end of this paper.

Davenport [2] is denoted a Van Slyke-Davenport diagram. In this diagram, the areas in which pH_p and $c^p_{HCO_3^-}$ may be expected to fall in normal conditions, primary respiratory and non-respiratory disturbances, compensated respiratory and non-respiratory disturbances and mixed disturbances are indicated. In addition, the diagram contains CO_2 isobars and CO_2 buffer lines. A CO_2 isobar is a set of points with equal P_{CO_2}; a CO_2 buffer line is a graph showing the change of pH_p and $c^p_{HCO_3^-}$ upon changes of P_{CO_2}. The basic ideas underlying the use of the diagram can be found elsewhere [1-3].

The diagram demonstrates that the diagnosis of an acid-base disturbance cannot be based on either pH_p or $c^p_{HCO_3^-}$ (or P_{CO_2}) alone. As Van Slyke put in 1921 [1]:

> In order to determine which one of the possible variations exists in the blood *in vivo*, it is necessary to ascertain two of the involved variables, such as the pH, $[BHCO_3]$, and $[H_2CO_3]$. With any of two of them a point can be located in its proper area on a diagram ..., but with any one of them alone it cannot be done.

The diagram obviates the use of so-called P_{CO_2} independent quantities (table 1) for the diagnosis of acid-base disturbances. For the following reason, these quantities are nevertheless widely in use. A respiratory disturbance of acid-base balance or a ventilatory compensation of a non-respiratory disturbance manifests itself in a change of P_{CO_2}. A non-respiratory disturbance of acid-base balance or a metabolic or renal compensation of a respiratory disturbance manifests itself in a change of $c^p_{HCO_3^-}$. However, whereas a respiratory disturbance and a ventilatory compensation of a non-respiratory disturbance can be assessed by comparing the actual P_{CO_2} with normal, non-respiratory disturbances, metabolic and renal compensations of respiratory disturbances cannot be assessed by simply comparing the actual $c^p_{HCO_3^-}$ with normal. This is because changes of P_{CO_2} also cause changes of $c^p_{HCO_3^-}$. Hence, a deviation of $c^p_{HCO_3^-}$ from normal may be due either to a non-respiratory disturbance or metabolic or renal compensation or to a respiratory disturbance or ventilatory compensation. The basic idea underlying the so-called P_{CO_2}-independent quantities is that the respiratory deviation of $c^p_{HCO_3^-}$ is ruled out by restoring a normal P_{CO_2} in blood or plasma *in vitro*. Figure 2 illustrates that this basic idea is not true. The course of pH_p with $c^p_{HCO_3^-}$ when P_{CO_2} is restored to normal in blood or plasma *in vitro* differs from that *in vivo*, *i.e.*, the course of the buffer line of isolated plasma and of blood *in vitro* both differ from that of blood *in vivo*. In the case of the primary respiratory acidosis of figure 2, standard bicarbonate is lower than normal. This would suggest a non-respiratory acidosis (complicating a prevailing respiratory acidosis). The same conclusion would be drawn if base excess or buffer base values were calculated from pH_p, $c^p_{HCO_3^-}$ and P_{CO_2}. Alkali reserve is higher than normal in the case of figure 2. This would suggest a metabolic or renal compensation of the respiratory acidosis.

It should be emphasized that neither the actual $c^p_{HCO_3^-}$ nor the alleged P_{CO_2}-independent quantities are an unequivocal measure for non-respiratory disturbances from which the amount of acid or base to be administered for restoring pH_p can be calculated [4,5]. This

Table 1. Definition and methods for the determination of so-called P_{CO_2} independent quantities used as a measure of the non-respiratory component of a disturbance of acid-base balance.

Quantity	Alkali reserve	Buffer base	Standard bicarbonate	Base excess
Definition	$c^p_{HCO_3^-}$ after separating plasma from blood cells and equilibrating with normal P_{CO_2} at normal temperature [8].	Total equivalent concentration of buffer anions in blood [9].	$c^p_{HCO_3^-}$ in fully oxygenated blood after equilibrating with normal P_{CO_2} at normal temperature [10].	1948: excess of buffer base over the normal buffer base being the buffer base at normal pH, P_{CO_2} and temperature [9] 1960: titratable base on titration to normal P_{CO_2} and temperature [11].
Analytical procedure	Centrifugation of blood and equilibration of plasma with endexpiratory gas; determination of $c^p_{CO_2}$; correction or $c^p_{CO_2}$ for free CO_2.	Determination of $pH_b(37)$, $c^b_{CO_2}$, c_{Hb} (or H or O_2 capacity); graphic determination of buffer base using the Singer Hastings nomogram of modifications thereof.	Equilibrium of blood with CO_2/O_2 gas mixture with P_{CO_2} = 40 mm Hg at 37 °C; determination of pH (37); graphic determination of $c^p_{HCO_3^-}$ using a log P_{CO_2}/pH diagram with HCO_3^- isopleths.	Equilibration of blood with two CO_2/O_2 gas mixtures with known P_{CO_2} at 37 °C; determination of $pH_b(37)$; graphic determination of base excess by plotting pH_b against P_{CO_2} in a pH_b/log P_{CO_2} diagram with preprinted base excess curve.
Derivation from $pH_b(37)$, $c^b_{CO_2}$, c_{Hb} and S_{O_2} using Brunsting's modification [12] of the Singer-Hastings nomogram.	Read buffer base at c_{Hb} = 0; read $c^p_{HCO_3^-}$ for P_{CO_2} = 40 mm Hg at buffer base found and c_{Hb} = 0.		Read buffer base at c_{Hb} measured; read $c^p_{HCO_3^-}$ for P_{CO_2} = 40 mm Hg at buffer base found and c_{Hb} measured.	Read buffer base at c_{Hb} measured; subtract buffer base read for $pH_b(37)$ = 7.40 and P_{CO_2} = 40 mm Hg at c_{Hb} measured from buffer base found.

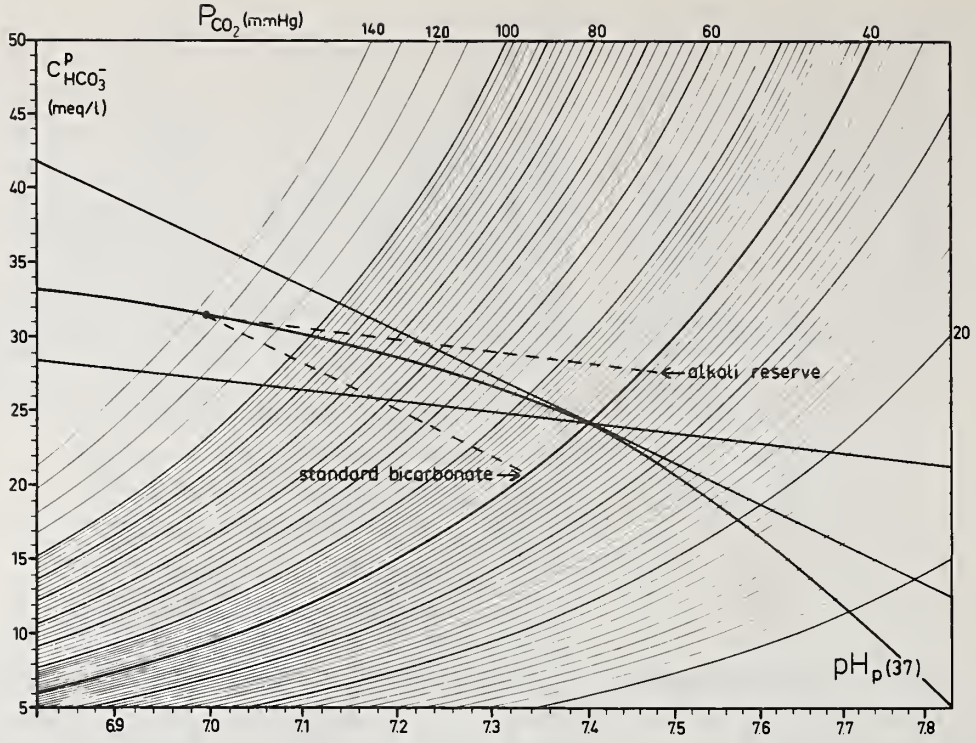

Figure 2. *Alkali reserve* and *standard bicarbonate* during a severe primary respiratory acidosis. The alkali reserve is seen to be higher than normal, the standard bicarbonate to be lower than normal.

is because changes of $c^p_{HCO_3^-}$ (and thus, changes of P_{CO_2}-independent quantities which are in some way related to or derived from $c^p_{HCO_3^-}$ (table 1)) are but a distant reflection of the non-volatile acids or bases actually gained or lost. In contrast to a respiratory disturbance where the change of P_{CO_2} is a direct measure of the acid gained or lost, a change of $c^p_{HCO_3^-}$ may indicate gain or loss of organic acids in metabolic disorders, gain or loss of inorganic acids or bicarbonate in renal disorders and gain or loss of bicarbonate or hydrochloric acid in gastro-intestinal disorders. It has been found, that the same pH_p and $c^p_{HCO_3^-}$ may occur while different amounts of non-volatile acids or bases have been gained or lost [6].

It should be also emphasized that neither the use of a pH_p-$c^p_{HCO_3^-}$ diagram nor the use of P_{CO_2}-independent quantities releases the physician who has to treat a patient with an acid-base disturbance, from the obligation to go deeply into the pathophysiology of this disturbance. The following case taken from reference 7 serves to illustrate this. It concerns a 45-year old female patient suffering from chronic obstructive pulmonary disease, admitted to the hospital during an acute exacerbation. On analysis of an arterial blood sample, pH_p = 7.17, $c^p_{HCO_3^-}$ = 33 meq·l^{-1}, P_{CO_2} = 92 mm Hg and S_{O_2} = 59 percent were measured. Treatment consisted of mechanical ventilation to bring $P^a_{CO_2}$ back to normal. A second arterial blood sample then yielded pH_p = 7.59, $c^p_{HCO_3^-}$ = 34 meq·l^{-1}, P_{CO_2} = 36 mm Hg and S_{O_2} = 96 percent. From acidemic, the patient had become alkalemic; the treatment thus

resulted in a serious condition leading to severe neurologic disfunction. Such mismanagement may easily result from the uncritical application of either a pH_p-$c^p_{HCO_3^-}$ diagram or of P_{CO_2}-independent quantities. Considering the patient's history, it may be assumed that before the acute exacerbation the patient had a compensated respiratory acidosis with high P_{CO_2}, high $c^p_{HCO_3^-}$ and a nearly normal pH_p, and with an arterial P_{O_2} still high enough to ensure an arterial S_{O_2} of about 90 percent. During the acute exacerbation P_{CO_2} increased and P_{O_2} decreased. The increase of P_{CO_2} would in itself cause an increase of $c^p_{HCO_3^-}$ (and a decrease of pH_p). However, P_{O_2} then decreased to values corresponding to the steep part of the O_2 dissociation curve and S_{O_2} decreased considerably. Hence, the patient became hypoxic, lactic acid accumulated, pH_p decreased further and $c^p_{HCO_3^-}$ decreased to a value usually found during a primary respiratory acidosis. However, in this patient with chronic respiratory disease a much higher $c^p_{HCO_3^-}$ was to be expected if her condition had not been complicated by a non-respiratory disturbance. When hypoxia was removed by mechanical ventilation, the accumulated lactic acid was metabolized and $c^p_{HCO_3^-}$ increased. As P_{CO_2} decreased far below the value existing before the acute exacerbation, pH_p increased to a high value.

If the patient's history had been considered with enough insight in the pathophysiology of acid-base disturbances, the mechanical ventilation would have been so regulated that a nearly normal pH_p would have been obtained instead of a normal P_{CO_2}. For such an insight, the alleged P_{CO_2}-independent quantities are not required.

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BASE EXCESS. WHY REOPEN THE ACID-BASE DEBATE?

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Base excess (BE) [1][1] was introduced as a formal mathematical concept with the Astrup system (AS) [2] in 1960, although the descriptive terms *base excess* and *base deficit* were used by Peters and Van Slyke in the 1930's [3]. By 1963, many criticisms had been leveled against it. Astrup himself [4] proposed a conference to discuss the many problems in acid-base understanding. The outcome was the 1964 New York Academy of Science Conference whose contents were published in 1966 [5]. We must take this conference as a baseline to see what progress has been made in the last decade. The Scandinavians conceded that BE was not a completely objective description of the whole body's acid-base state. Astrup indeed never claimed that it was; he held that it was an excellent way of describing changes in the blood, not the whole body [4]. However, some of his supporters still tried to apply the system to the whole body. In 1966, came the *"corrected base excess"* which was a formula to modify the BE in the light of knowledge about the way the *in vivo* CO_2 titration curve differed from the *in vitro* one [6]. This corrected base excess did not achieve wide popularity. As the decade passed the old arguments became half forgotten and peace reigned once more. In 1971, BE was modified to allow for buffering in EC fluid (SBE) and this was incorporated into the Siggaard-Andersen chart nomogram without too much fuss [7]. Then more recently, rumbles of the old acid-base debate have been heard again. Why is this and what do the rumblers hope to achieve?

Table 1. Recent milestones in acid-base history.

1954	Mark I Astrup equipment
1957	Standard bicarbonate
1960	Astrup trolley & system: base excess
1962	RIpH Moran Campbell
1963	Schwarz & Relman critique of Astrup system
1964	NYAS Conference: peace for a decade
1975/5	SI units. New analysers with old parameters

Table 2. Measurements of the non-respiratory component.

Plasma HCO_3^- :	Van Slyke 1917, 1931
Non-respiratory pH:	Hasselbalch 1916
	Whitehead 1964
	Stoker 1972
Buffer base:	Singer & Hastings 1948
Standard HCO_3^- :	Astrup 1957
Base excess:	Siggaard-Andersen 1960
Corrected BE:	Prys Roberts 1966
Standard BE:	Siggaard-Andersen 1971

[1]Figures in brackets indicate the literature references at the end of this paper.

The first and foremost point is that the instrument manufacturers have themselves brought various skeletons out of the closet on their current blood gas analysers. They appear increasingly reluctant to make simple machines that measure only pH, PO_2 and PCO_2. What they like to sell at greater profitability are larger machines with accessory electronic circuits to calculate and print-out or display many of the old derived parameters discussed at the 1964 Conference. The only modern parameter seems to the SBE on the Radiometer ABL1 and of course this has been attacked on the familiar arguments that there is no way of knowing what the ECF buffers are in any particular patient, especially one with an acute on chronic acid-base disturbance.

Table 3. Parameter measured on some current instruments.

	Blood Gas Analysers			
Name:	Radiometer ABL	Corning Eel 165	IL 413	Sandoz AVL 937
Measures:	Hb conc.	All measure pH, PCO_2 & PO_2		
Calculates:	P-HCO_3^-	P-HCO_3^-	P-HCO_3^-	P-HCO_3^-
	Std HCO_3^-		Std HCO_3^-	
	total CO_2	total CO_2	total CO_2	total CO_2
	base excess	base excess	base excess	base excess
	Std base excess			buffer base
	O_2 saturation			O_2 saturation

The second major reason for the re-opening of the debate is the growing use of SI units in chemical pathology. This process is gaining momentum in Europe; for example, SI units will be in widespread use in the UK by the end of 1975 [8]. With the introduction of the SI, it is convenient to ask ourselves whether H^+ concentration can be more conveniently reported in an arithmetic SI unit, *i.e.*, nanomole/liter, as compared with the logarithmic pH notation. Of course these arguments are anything but new. It is worth pointing out that the pH notation was introduced by Sørensen as a shorthand for the otherwise unwieldy H^+ concentration scale [9]. Arguments about what electrodes actually measure, and activity versus concentration arose soon after. For many years H^+ activity was reported by the special symbol pαH and it was only after Peters and Van Slyke, in their classic work published in 1931, pronounced in favor of the use of pH to cover both H^+ activity *and* concentration that this special symbol was finally abandoned [3]. In more recent years, many individual workers have made use of the simpler arithmetic scale. One classic plea was Moran Campbell's RIpH in 1962 [10]. The upheaval caused by the introduction of the SI provides us with a very convenient opportunity to make this change. The arguments will remain, but at least those who wish to use the arithmetic SI unit will find it easier to get their colleagues to accept the change. Already some gas analysers have partial pressures in kilopascals and no doubt soon we will be able to get an arithemtic $[H^+]$ output by means of an antilog circuit board as used with ion-selective electrodes for ions other than the H^+.

Further reasons for the fresh debate are a little more nonspecific. It is partly due to a rejection of the rather Teutonic heavy-handed approach of the Astrup system with its formulae and nomograms by a younger generation of clinicians. They are often unaware of the emotions generated in the past by the debates and they prefer a more flexible approach to acid base assessment. Some points are:

A. A growing suspicion of nomograms because the historic need for them has largely gone. They were needed in the past because laboratory instrumentation lagged behind theory of acid-base understanding for many years. Thus, nomograms were introduced to extrapolate the scanty laboratory data, *e.g.*, to predict pH from CO_2 measurements. With the availability of ion-selective electrodes the need for nomograms has gone.

Table 4. Why there used to be a need for nomograms.

(1) Need to estimate blood H^+ concentration, *e.g.*, from plasma CO_2 and HCO_3^-

(2) Assumed that physio-chemical constants apply to biological systems:
CO_2 solubility coefficient = 0.510
pK_1^I = 6.10 ± 0.01.

B. The modern approach is thus that laboratories should only report what they actually measure and leave it to the individual clinician to carry around the Radiometer blood gas calculator [11] if he wishes to derive parameters from the primary data. At a higher level of sophistication we are of course still in trouble. A CO_2 electrode does not really measure PCO_2 but a pH change related, we hope, to it. What do osmometers really measure? How are they calibrated?

Table 5. Philosophy of the modern approach.

(1) Clinical chemists should report only:
what is actually measured
what is usefully measured, *e.g.*,
blood $[H^+]$, P_aCO_2, actual HCO_3^-.

(2) They should not report derived parameters, *e.g.*, PCO_2 at 33° when measured at 38°.

(3) Use of nomograms should be restrained.

C. Thirdly, there is a greater interest in the interrelationships between O_2 delivery to the tissues and H^+ concentration. Hence, more measurements are needed in acid-base work than the big three (pH, PO_2, PCO_2). When we talk of the P_{50} we enter another nomogram area because it is still a relatively new topic. Since the O_2 dissociation curve is pH-dependent the P_{50} would be best measured at the *in vivo* pH. One group at NIH calculates the so-called *in vivo* P_{50} from the standard pH 7.40 P_{50} using the MCHC, DPG, BE, pH and temperature [12]. So far we have restricted ourselves to measure red cell DPG as µmol/g Hb (not per liter red cells because the cells alter their shape in acidosis and alkalosis).

Table 6. Summary of current acid-base tests.

1974	1975
pH	H^+ conc. nmol/l
PCO_2 mmHg	PCO_2 kPa
P-HCO_3^- meq/l	P-HCO_3^- mmol/l
Std P-HCO_3^- meq/l	PO_2 & P_{50} kPa
Base Excess MkI-III	2:3-DPG
PO_2 mmHg	

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USE OF *IN VIVO* CO_2 TITRATION CURVES IN THE PHYSIOLOGICAL ASSESSMENT OF ACID-BASE BALANCE

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In their 1963 critique of the Astrup system, Schwartz and Relman [1][1] recommended instead the use of a physiologic approach to the clinical assessment of acid-base balance in individual patients. This approach was based upon the interpretation of clinical and laboratory data in the light of the known behavior patterns in respiratory and metabolic disease. This approach can be updated by making use of the *in vivo* CO_2 titration curves in man. Assessment can then be subdivided into three broad areas:

1. Primary acid-base data

   a. The respiratory component - arterial blood Pco_2.

   b. The acidity of blood--H^+ nmol/l (or pH) which gives direct information about the accumulation of non-volatile acid or base and indirectly of changes in Pco_2.

2. Other clinicopathological information

   a. Clinical data such as the duration and severity of the patient's present illness, its antecedent pathological conditions (if any), and treatment which might modify acid-base status.

   b. Laboratory data such as plasma actual HCO_3^- and other appropriate measurement, *e.g.*, arterial blood Po_2, P_{50} (partial pressure of O_2 when Hb is 50 percent saturated with O_2), red cell DPG, K^+ and concentration of other electrolytes.

3. Knowledge of the following:

   The usual compensatory changes to altered arterial blood H^+ and Pco_2:

   i. The ventilatory response to metabolic acidosis and alkalosis.

   ii. The renal response to respiratory acidosis and alkalosis.

In the physiological assessment of acid-base balance, it is important to know how the body reacts to acute and chronic changes in the two primary measurements, arterial blood $[H^+]$ and Pco_2. The relative changes in $[H^+]$ and Pco_2 are different in metabolic and respiratory disturbances since the ventilatory response to metabolic acidosis and alkalosis is rapid whereas the renal response to respiratory failure (acidosis) is slower, taking 3-4 days at least to become maximal.

1. The Ventilatory Response to Changes in $[H^+]$ in Metabolic Acidosis and Alkalosis

When the $[H^+]$ in the blood changes abruptly the Pco_2 may not alter, but within a few hours there is a ventilatory response to changes in blood $[H^+]$ which may take 24 hours or

[1]Figures in brackets indicate the literature references at the end of this paper.

more for the compensatory change in Pco_2 to be complete [2]. In compensated metabolic acidosis, it is rare for the arterial blood Pco_2 to fall below 2.0 kPa (15 mmHg) and the Pco_2 never falls below 1.33 kPa (10 mmHg) as the work of breathing limits the maximum ventilation volume.

In metabolic alkalosis, the suppression of normal ventilation is similarly a compensating mechanism. Observations of patients with arterial blood [H+] varying from 30 to 130 nmol/l (pH 7.55-6.88) show that there is a hyperbolic relationship between the $[H^+]$ and Pco_2 [3,4].

2. The Renal Response to Changes in Pco_2; Respiratory Acidosis and Alkalosis

When the arterial blood Pco_2 is acutely altered either by changes in ventilation or breathing artificially high CO_2 mixtures, the resulting acid-base values are known as the whole body CO_2-titration curves. They have been used for a number of years in the assessment of acute respiratory acidosis [5]. The blood $[H^+]$ values normally found as the blood Pco_2 changes from 2.0 to 12.0 kPa (15-90 mmHg) are shown in figure 1 [6,7]. The body reacts to a

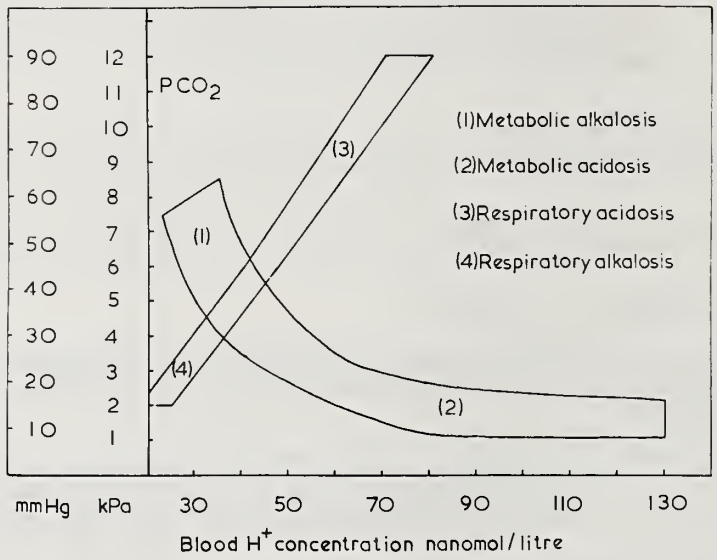

Figure 1. Blood H^+ concentration and Pco_2 chart showing the range of observed values during primary compensated metabolic alkalosis and acidosis and acute uncompensated primary respiratory alkalosis and acidosis. During primary metabolic changes, there is a superimposed respiratory response. (Data from Bone, *et al.* [3] and Fulop, *et al.*[4] for the metabolic changes, and from Arbus, *et al.*[7] and Brackett, *et al.* [6] for the respiratory changes.)

raised Pco_2 by the renal retention of HCO_3^- and excretion of H^+. This process is significant during the first 24-48 hours and becomes maximal after 3-4 days [8]. If the arterial blood Pco_2 remains below about 8 kPa (60 mmHg), the blood $[H^+]$ may remain near normal, but renal compensation begins to fail when the Pco_2 rises above 9.5-10 kPa (71-75 mmHg) [9,10]. An acute rise in arterial blood Pco_2 is invariably accompanied by a fall in arterial blood Po_2 which may be severe enough to cause lactic acidosis. In respiratory failure without assisted ventilation, it is rare for the Pco_2 to rise above 10.5-12 kPa (79-90 mmHg) as the associated hypoxemia (Po_2 4-2.6 kPa) (30-20 mmHg) endangers life [11].

Hyperventilation may cause an acute fall in arterial blood Pco_2, the plasma HCO_3^- may not change significantly, but the $[H^+]$ may become dangerously low. The changes found during inhalation of low O_2 gas mixtures and during adaptation to high altitudes are similar; the renal adaptation to a low arterial blood Pco_2 is slow and may take several weeks for completion [12].

If a high arterial blood Pco_2 is reduced by artificial ventilation, the restoration of normal acid-base balance is slow because of the time taken for the renal compensating process to work in the opposite direction. The kidneys may continue to excrete an acid urine for some time, thereby producing a dangerous acid-base state indistinguishable from primary uncompensated metabolic alkalosis [13].

3. The Whole-Body *in vivo* CO_2-Titration Curves

When patients with different initial levels of $[H^+]$ (pre-existing metabolic acidosis or alkalosis) are ventilated, the resulting *in vivo* CO_2-titration curves are almost parallel to the normal response. Hence, it has been suggested that the *in vivo* whole-body CO_2-titration curves be used as an aid to the interpretation of acid-base problems [14-17]. Figure 2 shows whole-body *in vivo* CO_2-titration figures from the work of Stoker and his colleagues [14] recalculated and plotted out in SI units [18].

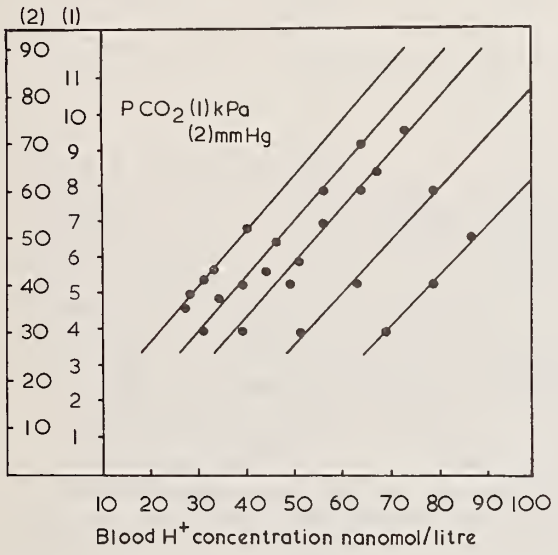

Figure 2. Whole-body CO_2 titration lines *in vivo* in man at different values of nonrespiratory H^+ Concentration (blood H^+ at Pco_2 5.3 kPa (40 mmHg)). (Data from Stoker, *et al.* [14].)

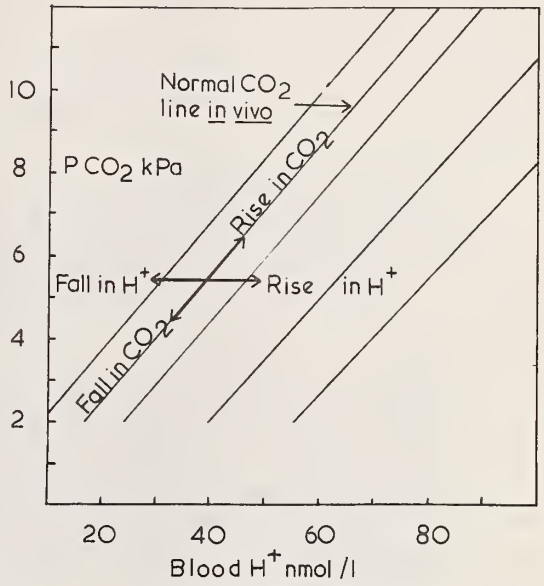

Figure 3. Whole body CO_2 titration lines *in vivo* in man as shown in figure 2 with points omitted to show the effects on Pco_2 of acute changes in H^+ concentration (rise and fall in H^+) and on H^+ concentration of acute changes in Pco_2 (rise or fall in Pco_2).

When a patient is ventilated or retains CO_2 acutely, the points representing $[H^+]$ and Pco_2 move along a straight line parallel to those in figure 3 in which the individual points have been omitted for clarity. An acute metabolic rise or fall in blood $[H^+]$ causes a horizontal movement to the right or left. Mixed and compensated acid-base disturbances show movement resulting from both vectors.

4. Examples of the Use of the *in vivo* Chart

The use of the *in vivo* chart will be illustrated by some examples. It should be noted that the chart is not a nomogram in that it is not used to calculate derived parameters. The purpose of the chart is to simplify analysis of acid-base data by comparing the behavior of the individual patient with the known behavior in metabolic and respiratory change.

A. Acute metabolic acidosis during chronic respiratory acidosis

Even apparently simple acid-base disturbances can have a "mixed" component. Figure 4 shows how an acute exacerbation of hypoxemia in chronic respiratory failure during which period the blood Pco_2 did not alter caused a "type A" lactic acidosis [19]. There was a horizontal "shift to the right" of H^+ and the HCO_3^- fell from 38 to 30 mmol/l. This severe

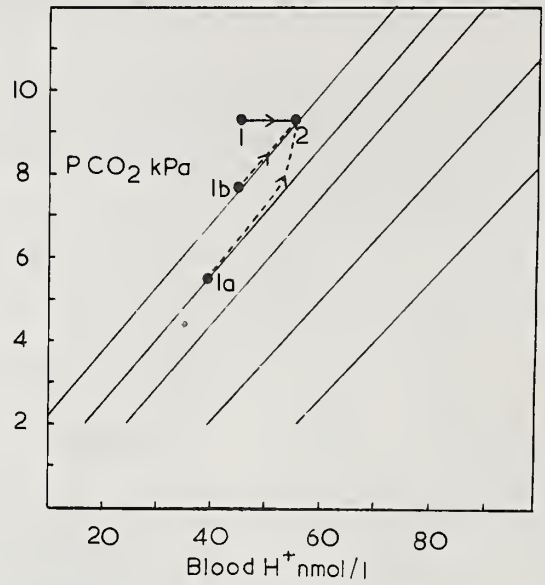

Figure 4. Acute metabolic acidosis due to worsening of hypoxia during chronic respiratory failure. The arterial blood Po_2 fell from 4.7 kPa (35 mmHg) at point (1) to 2.7 kPa (20 mmHg at point (2) and the bicarbonate fell from 38 mmol/l, point (1) to 30 mmol/l at point (2) without change in Pco_2 (9.3 kPa (70 mmHg)). The primary acid-base results at point (2) could equally have arisen from an acute respiratiry acidosis with early renal compensation in a previously normal individual (point 1a to 2), or from an exacerbation of CO_2 retention in a patient with moderate chronic respiratory acidosis (point 1b to 2).

chronic respiratory acidosis, with maximal renal compensation, and the additional acute metabolic acidosis cannot be distinguished from inspection of the blood acid-base results from an acute severe respiratory acidosis with early renal compensation in a previously normal individual (point 1a) or an acute exacerbation of CO_2 retention in a patient with moderate chronic respiratory acidosis (point 1b).

B. Acute on chronic respiratory failure

Figure 5 shows the changes over 48 hours in a patient with an acute exacerbation of chronic bronchitis. On admission (1), the arterial blood Pco_2 was acutely elevated. Despite antibiotics and controlled O_2 administration, there was further CO_2 retention. Intermittent positive pressure respiration for 24 hours reduced the arterial blood Pco_2 to 8.0 kPa (60 mmHg) which was similar to the patient's normal Pco_2.

C. Acute respiratory acidosis with concealed metabolic alkalosis

A woman aged 60 years was admitted in acute respiratory failure; she was confused, making a history impossible, restless and cyanosed. Intermittent positive pressure ventilation was instituted and after a few hours, ventilation results were at point (2) (see fig. 6). At this point, it is now clear that the patient probably had a mixed disturbance since, with the correction of the respiratory failure by intermittent positive pressure

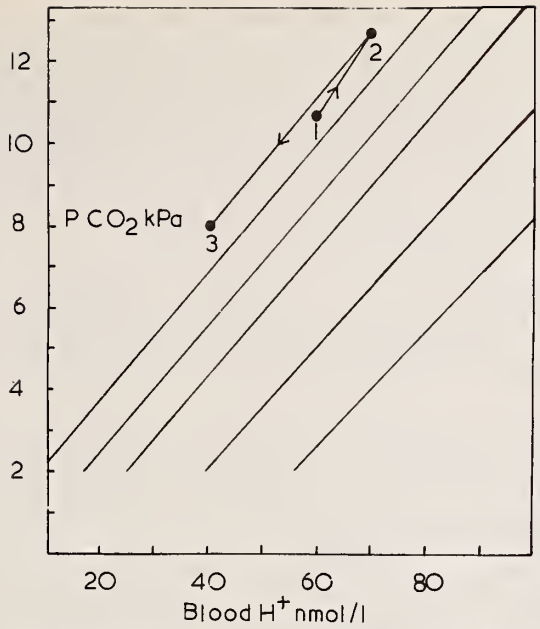

Figure 5. Acute exacerbation of chronic bronchitis in a man aged 65 years. On admission (1), the patient was hypoxemic, Po_2 4.0 kPa (30 mmHg), plasma HCO_3^- 32 mmol/l; (2), further increase in CO_2 retention during early treatment, plasma HCO_3^- 36 mmol/l. Intermittent positive-pressure ventilation was instituted which rapidly lowered the Pco_2 (3), plasma HCO_3^- 38 mmol/l, but the patient remained in compensated respiratory failure.

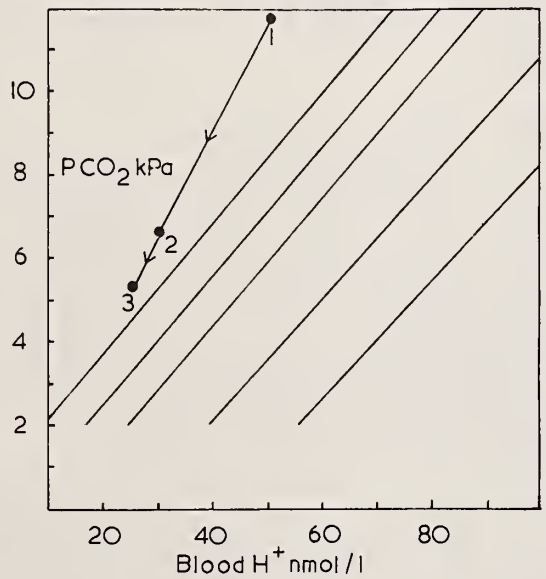

Figure 6. Acute respiratory failure with concealed metabolic alkalosis in a woman aged 60 years. On admission, the patient was cyanosed and comatose; Po_2 6.4 kPa (48 mmHg), plasma Na^+ 137, K^+ 5.8, Cl^- 83, HCO_3^- 34, urea 3.3 mmol/l (20 mg/dl). Intermittent positive pressure respiration quickly reduced the Pco_2 and revealed the metabolic alkalosis (point 2) which was subsequently found to be associated with long-term diuretic therapy for heart failure due to chronic hypoxemia with bronchitis. At point (3), electrolyte results were plasma Na^+ 140, K^+ 2.7, Ca^{2+} 1.85 (7.4 mg/dl), HCO_3^- 45 mmol/l and the patient began to have tetanic convulsions due to the untreated metabolic alkalosis from which she died 48 hours later.

respiration, her results at point (2) lie in the area of metabolic alkalosis shown previously. One can thus easily predict that more ventilation would only lower still further the already dangerously low [H^+]. Unfortunately, ventilation was continued and the patient died after 48 hours from tetanic convulsions. The full history later obtained from relatives revealed that the patient was on long-term diuretic therapy for congestive cardiac failure due to chronic hypoxemia with air way obstruction. The typical hypokalemia was concealed initially by the respiratory acidemia. Thus, the patient had two separate reasons to develop a metabolic alkalosis: (a) urinary K^+ loss due to diuretic therapy, and (b) paradoxical aciduria after lowering the high Pco_2 (see above).

In retrospect, it is evident that this patient's only chance was for energetic treatment of the metabolic alkalosis at point (2). Intravenous HCl is direct and logical [20]. If given stronger than 0.09 mol, it may cause hemolysis and intravenous sodium phosphate, ammonium chloride, arginine hydrochloride, and even dialysis have been advocated.

There has been current interest in metabolic alkalosis because its incidence has been reported to be rising and the body tolerates alkalosis much less well than acidosis [21]. Whereas patients have survived with blood [H^+] 170 nmol/l (pH 6.78), the mortality is high in metabolic alkalosis with blood [H^+] 28 nmol/l (pH 7.55) and survival is rare below blood [H^+] 23 nmol/l (pH 7.65). Alkalosis may occur not only from potassium loss and in hepatic failure, but also after surgery, *e.g.*, after bypass operations, and in trauma, especially associated with sepsis and peritonitis. The causes are varied and may include: loss of H^+ through gastric suction; over-use of alkaline liquids in early therapy, or urinary potassium loss through adrenal cortical hormone activity [21].

Table 1. Metabolic alkalosis.

Mortality rises with fall in [H^+]:

moderate	$H^+ < 28$ nmol/l	pH 7.55
high	$H^+ < 23$ nmol.l	7.65

Occurrence:

Medical: drugs; *e.g.*, diuretics
acute liver failure
chronic bronchitics on ventilation

Surgical: major trauma, by-pass operations
sepsis, hemorrhage, peritonitis

Aetiological Factors:

loss of gastric H^+ - suction
alkaline fluids - ACD blood, $NaHCO_3$
paradoxical aciduria after fall in Pco_2
K^+ loss in urine - adrenal hormones

Treatment

0.09 mol HCl in NaCl
iv NH_4Cl
iv Na phosphate, pH 7.0
iv arginine, HCl

In view of the large iatrogenic component and the graver risk to life in metabolic alkalosis as compared with metabolic acidosis, it is clear that physicians and anesthetists working in intensive care units need to exercise special care to prevent the unnecessary development of metabolic alkalosis.

D. Compensated metabolic acidosis

Figure 7 shows the acid-base changes over 12 hours during the resuscitation of a patient with acute diabetic ketoacidosis. On admission (1), there was a marked metabolic acidosis with compensatory low Pco_2. Therapy with insulin, intravenous NaCl and potassium supplements was enough to bring the patient to near normal acid-base balance. Intravenous $NaHCO_3$ was not given.

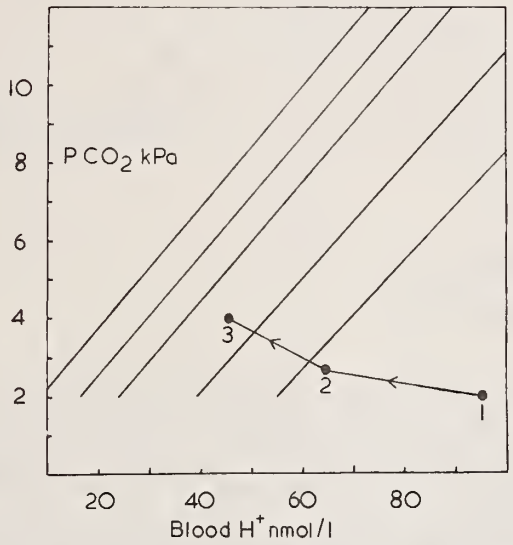

Figure 7. Acute diabetic ketoacidosis in a man aged 30. On admission, the plasma glucose was 20 mmol/l (360 mg/dl), plasma HCO_3^- less than 5 mmol/l. The patient was resuscitated with intravenous NaCl with KCl supplements and insulin. After 6 hours (2), the plasma HCO_3^- was 8 mmol/l. The patient had recovered by 12 hours (3), although the acid-base state was not completely normal, plasma HCO_3^- 18 mmol/l.

E. Compensated metabolic alkalosis and acute respiratory acidosis

A further example of a complex acid-base disturbance was provided recently (figs. 8 and 9). A 45-year-old workman (stone mason) was admitted in a semi-comatose state, peripheral cyanosis, vomiting and tetany. From his past history of suspected alcoholic excesses, he was thought initially to have possibly Wernicke or hepatic pre-coma. His LFTs, blood EtOH and red cell TK did not support this view so he was urgently re-assessed.

Date	14	15	16	17	21	22 April 1975
H^+		23	36	36	37	35 nmol/l
Na^+	132	140	137	145	138	134 mmol/l
K^+	2.6	3.1	3.9	3.1	3.4	2.7 mmol/l
HCO_3^-	-	>50	>40	35	35	22 mmol/l
Urea	15.5	27.4	25.0	15.6	4.5	4.2 mmol/l
PCO_2		10.0	6.7	6.1	6.9	5.1 kPa
PO_2		5.9	10.3	8.1	12.6	9.8 kPa
DPG		31.9	25.3	13.9	14.1	15.6 µmol/g Hb

14th: semi-coma, cyanosis.?liver/EtOH
15th: intubated and ventilated until 21st
16th: pneumothorax:chest drained
Δ(1) chronic DU.Alkalosis from oral $NaHCO_3$
(2) RLLpneumonia

Figure 8. Clinico-pathological data (table) on a man aged 45 with combined chronic metabolic alkalosis due to ingestion of $NaHCO_3$ and acute respiratory failure due pneumonia and pneumothorax.

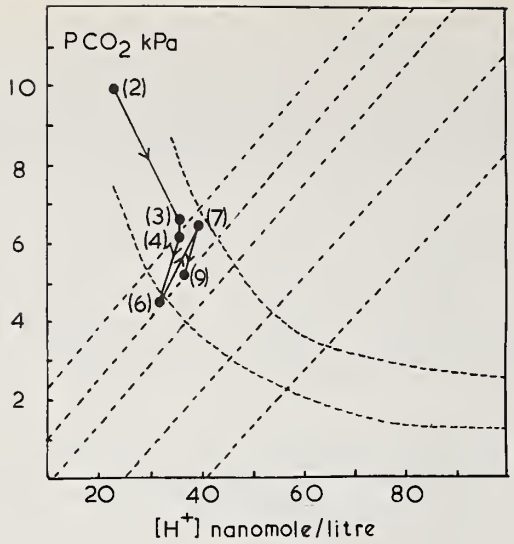

Figure 9. Primary acid-base results show days after admission in brackets.

A gross metabolic alkalosis, right lobar collapse and pneumothorax were found and he was intubated and ventilated. The alkalosis was due to excess ingestion of $NaHCO_3$ for the pain of a chronic DU. The initial blood gas results are thus consistent with a profound compensated metabolic alkalosis and the high red cell DPG results support this.

The acid-base disturbance was fairly rapidly restored to near-normality, but then his acid-base point moved up and down the *in vivo* line a bit as is often found in patients on ventilation. There was thus a chronic condition with an acute respiratory episode which led to the patient's hospitalization.

F. Combined metabolic and respiratory acidosis

In cardiac arrest, there is a combination of acute respiratory acidosis and hypoxic metabolic acidosis. Figure 10 shows the sequence of events in the attempted resuscitation of a man aged 55 who arrested after a myocardial infarct. Blood taken 15 minutes after the arrest showed a profound combined acidosis. The Pco_2 was reduced by ventilation (2) and the metabolic component was improved by 100 mmol of intravenous $NaHCO_3$ (3). At this point, the airway became obstructed and after fruitless attempts to improve ventilation the patient died (5) about 2 hours after the arrest.

It is reasonable to consider reducing a high metabolic $[H^+]$ in life-threatening situations by administering $NaHCO_3$, although it is likely that death in severe acidosis is due to hypoxic damage rather that to H^+ ions *per se*. The arguments against $NaHCO_3$ therapy are:

1. Most acute metabolic acidosis is due to oxidizable acids:
   a. Lactic/Pyruvic acid in acute hypoxia.
   b. Free fatty, acetoacetic and β-hydroxybutyric acids in acute diabetic ketoacidosis.
2. O_2 release to the tissues is partly controlled by red cell 2,3-diphosphoglycerate (DPG) [22,23] which modifies the shape of the O_2-dissociation curve. For blood with normal O_2 affinity (P_{50} = 3.6 kPa (27 mmHg)), a "shift to the right" of 0.4 kPa (3 mmHg) may deliver over 20 percent more O_2 to the tissues. Hypoxia and $[H^+]$ are the two major physiological factors which, with plasma inorganic phosphorus, determine the red cell content of DPG.

Acidosis both improves O_2 release in the tissues (Bohr effect) [24] and depletes red cell DPG. Restoration of red cell DPG is a slow process ($T_{1/2}$ about 11 hours) and too hasty correction of acidosis allows the DPG deficiency to predominate by shifting the O_2-

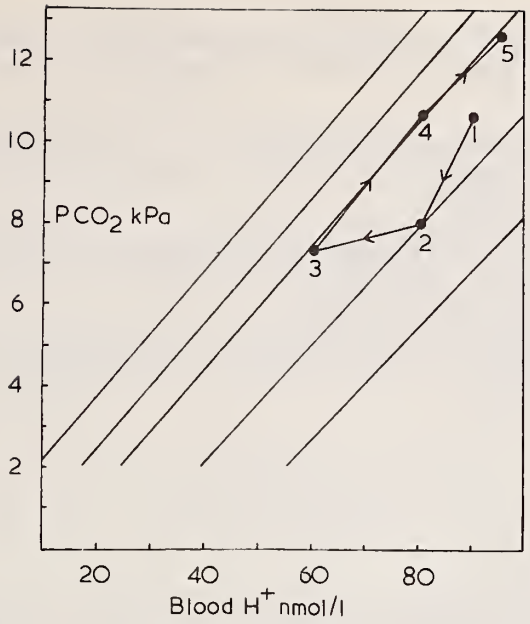

Figure 10. Attempted resuscitation of a man aged 55 years with cardiac arrest due to cardiac arrhythmia after a myocardial infarct. Results at point (1) taken on blood 15 minutes after the arrest showed a profound combined respiratory and metabolic acidosis, plasma HCO_3^- 18 mmol/l. The Pco_2 was reduced by ventilation (2) and the metabolic acidosis was improved after infusion of 100 mmol $NaHCO_3$ intravenously (3), plasma HCO_3^- 22 mmol/l. Further acute CO_2 retention supervened (4) and the patient died within 2 hours (5).

dissociation curve to the left, which impairs O_2 delivery to the tissues. Some workers have suggested correction of acidosis only to about $[H^+]$ 55 nmol/l (pH 7.26) [25] while others have advocated intravenous sodium phosphate so as to increase the availability of phosphorus for DPG production [26]. Equally, methods for the enzymatic determination of red cell DPG are improving [27] so that it is now possible to measure DPG routinely in patients with acute acid-base disturbance.

I am obliged to the editor of the *British Journal of Chest Diseases* for permission to make use of material published previously [17].

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RELATIVE ACTIVITY AND SI UNITS

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1. SI-Units

The name International System (Système International, SI) of Units was adopted by the 11th Conférence Générale des Poids et Mesures (CGPM) in 1960 [1][1]. The system is now based on seven base units for the corresponding basic kinds of quantities (table 1).

Table 1. The seven base units of the International System of Units. (Symbols for quantities are printed in italics, symbols for units in Roman type).

Quantity	Symbol	Dimension	Unit	Symbol of unit
length	l	L	metre	m
mass	m	M	kilogram	kg
time	t	T	second	s
electric current	I	I	ampere	A
thermodynamic temperature	T	Θ	kelvin	K
luminous intensity	I_v	J	candela	cd
amount of substance	n	N	mole	mol

All other SI units are derived from base units by multiplication or division without the introduction of numerical factors; for example the derived, coherent unit of volume is the cubic metre (m^3) and the derived, coherent unit of (substance) concentration is the mole per cubic metre (mol/m^3 or $mol \cdot m^{-3}$). Several derived SI units have been given special names, some of them are to be found in table 2.

The litre (l) is a non-coherent unit of volume. The litre has been redefined [2] and is now exactly equal to the cubic decimetre (dm^3). All other units containing the litre in either the numerator or denominator are non-coherent, *e.g.*, mol/l as the unit of substance concentration.

[1]Figures in brackets indicate the literature references at the end of this paper.

Table 2. Some derived SI units with special names. (A full stop (·) indicates multiplication of units. A solidus (/) indicates division of units; alternatively the denominator may be expressed with a negative exponent.)

Quantity	Symbol	Unit	Name	Symbol
force	F	$kg \cdot m/s^2$	newton	N
pressure	p	N/m^2	pascal	Pa
work, energy	W	$N \cdot m$	joule	J
		$Pa \cdot m^3$		
		$kPa \cdot l$		

An extremely important feature of the International System is the fact that there is only one unit of energy, the joule ($1\ J = 1\ N \cdot m = 1\ kg \cdot m^2/s^2$). Work is mechanical energy and, therefore, the unit of work is also the joule. The product of pressure (p) and volume (V) has the dimension of work; coherent units are pascal ($Pa = N/m^2$), cubic metre (m^3) and $Pa \cdot m^3 = N \cdot m = J$. When the litre is chosen as the non-coherent unit of volume, the kilopascal (kPa) must be the noncoherent unit of pressure [3].

$$1\ kPa \cdot l = 10^3\ Pa \cdot 10^{-3}\ m^3 = 1\ Pa \cdot m^3 = 1\ N \cdot m = 1\ J \tag{1}$$

2. Reference Quantities

The International Organization for Standardization defines a reference quantity in the following way [4]:

"Physical quantities are concepts used for qualitative and quantitative descriptions of physical phenomena. Such quantities may be classified into categories, each category containing only quantities which are mutually comparable. If one of the quantities in such a category is chosen as a reference quantity, called the *unit*, any other quantity in this category can be expressed as a product of this unit and a number, called the *numerical value* of the quantity. For a quantity symbolized by A, this relationship may be expressed in the form

$$A = (A) \times [A] \tag{2}$$

where $[A]$ is here used to symbolize the unit chosen for the quantity A, and (A) to symbolize the numerical value of the quantity A when expressed in the unit $[A]$."

The simplest case is present, when the unit is unity (proposed symbol I, not yet accepted by CQUCC and EPQU [5]). Examples are: volume fraction (ϕ) and mole fraction (x). The SI units for plane angle and solid angle, the radian (rad) and the steradian (sr), respectively, are called supplementary units in the International System of Units.

When a quantity has to be made dimensionless, two ways are followed.

a) The quantity A is divided by the unit $[A]$, thus giving the numerical value (A)

$$\frac{A}{[A]} = (A). \tag{3}$$

This procedure is recommended by Siggaard-Andersen [6,7].

b) The quantity A is divided by a quantity $A_o = 1$, representative for the standard state

$$\frac{A}{A_o} = \frac{(A)\cdot[A]}{(1)\cdot[A]} = (A) \ . \tag{4}$$

This convention is advocated by Bates [8].

In both cases the result of the operation is a numerical value depending on the unit chosen. This is in contrast with true dimensionless quantities, where the value is independent of the system of units, the unit being unity.

c) Theoretically--and why not practically--the solution is to refer to a standard quantity

$$\frac{A}{A_S} = \frac{(A)\cdot[A]}{(A_S)\cdot[A]} = (A/A_S) \ . \tag{5}$$

In this way a ratio of numerical values is obtained, this ratio being independent of the system of units.

3. Relative Activities

There are three different activity scales:

a) on a concentration (c) basis

$$a_c = y\cdot c \qquad \lim_{c=o} a_c/c = 1 \tag{6}$$

b) on a molality (m) basis

$$a_m = \gamma\cdot m \qquad \lim_{m=o} a_m/m = 1 \tag{7}$$

c) on a mole fraction (x) basis

$$a_x = f\cdot x \qquad \lim_{x=o} a_x/x = 1 \tag{8}$$

Activities on the molality and concentration scales are related by the equation [8]:

$$a_c/a_m = \rho_o \tag{9}$$

where ρ_o is the density of the solvent. When the solvent is water, the *value* of ρ_o "is very close to unity, and for this reason no distinction is made between a_c and a_m" [6].

The value of ρ_0 is close to unity only if the litre is chosen as the non-coherent unit of volume. The coherent SI unit of density is kg/m^3, the value of the density of water becomes approximately 1000. Therefore, the simplification is not justified.

When activities have to be made dimensionless, the suggestion of section 2.c. may be followed. For each scale there will be one standard quantity, but the relationship between these quantities will be known.

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SEMI-EMPIRICAL ACID-BASE PROGRAM

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In spite of many efforts (*e.g.*, [1-3][1]), mathematical models of the acid-base behavior of human blood have not yet led to useful programs for calculating derived acid-base data from measured primary data. The calculations of Scheffner and Martin [4] are restricted to the interpolation method for pCO_2 and the determination of actual bicarbonate, total CO_2 and standard bicarbonate.

On the other hand, empirical approaches have been quite successful. Siggaard-Andersen [5] distinguishes between programs utilizing linear pH, log pCO_2 equilibration curves and those utilizing linear pH, $ctHCO_3^-$ equilibration curves. We confine ourselves to the first category.

Dell and Winters [6] store the coordinates of the base excess and buffer base curves in the computer. Hardt [7] uses three different empirical equations for base excess, each working in its own pH area. Recently the same system was followed by Knoll *et al.* [8]. Vallbona *et al.* [9] apply a simplified equation for the calculation of the base excess concentration. Englesson *et al.* [10] make use of an assumed "mathematical center," the point of convergence for the pH-log pCO_2 lines.

We call our program a semi-empirical one, because the basic equations have a sound theoretical background, but are empirically adjusted to measured data by the introduction of second-order correction terms or factors.

The slopes of the equilibration lines of separated human blood plasma in the pH-log pCO_2 diagram could be predicted [11]. The same type of equation was used to describe the slopes of the equilibration lines of whole blood as a function of plasma protein, hemoglobin (Hb) and standard bicarbonate (SB) concentrations. Adding a term with Hb^2 led to the necessary accuracy for the calculation of Hb from a given slope M

$$M = -1.0285 + 0.0051\ Hb - (3.5242 + 0.6105\ Hb + 0.009\ Hb^2)/SB$$

Base excess (BE) is calculated from Hb content and Van Slyke standard bicarbonate (VB)

$$BE = (1 + D \times Hb) \cdot (VB - 24) \qquad (1)$$

[1]Figures in brackets indicate the literature references at the end of this paper.

with

$$D = - 0.0233(BE + 20)/(34.232 + BE) \quad (2)$$

instead of taking a constant value of D [5].

For further details of the program refer to [12].

The semi-empirical approach combines high accuracy with low computer power and could be used as a means of averaging measured data.

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"ACID-BASE SEMANTICS"--A CENTURY OF THE TOWER OF BABEL[1]

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Almost 100 years have passed since 1877 when Walter [3][2] originated the concept of *"alkaline reserve"* which was estimated by the *carbon dioxide content* of the plasma. It was assumed that the alkali of the blood existed mainly in the form of *carbonates* (or carbonates and bicarbonates as the terms are used today) and that the quantity of carbon dioxide was essentially proportional to the quantity of alkali contained in the blood. Walter [3] stated that "...the carbon dioxide content of the blood will...permit of conclusions as to the quantity of alkali that has been withdrawn from the body as a whole."

Subsequently, alkali(ne) reserve has been used not only to designate the total carbon dioxide content of blood but also the carbon dioxide capacity, the plasma bicarbonate (determined by the carbon dioxide *combining* capacity or carbon dioxide *combining* power), the amount of sodium combined with the plasma bicarbonate, and the total cation ("base") concentration. In 1931, Cummer [4] wrote that the alkali reserve "...by means of which acids are neutralized, is made up of the bicarbonates, small quantities of phosphates, and alkaline protein compounds."

The four "CO_2" determinations mentioned above are compared to each other and to the AutoAnalyzer^R technic in table 1. Skeggs placed the AutoAnalyzer trays in a box to equilibrate the plasma [5] or serum [6] specimens with carbon dioxide to obtain the CO_2 combining power since it was difficult to achieve the necessary anaerobic conditions during collection and analysis required for the CO_2 content. A modification was described in which each cup was equilibrated three times just prior to aspiration into the apparatus [7]. The present technic for the AutoAnalyzer CO_2 is termed a "content" since no equilibration is utilized but, in reality, the result is an *approximation* of a bicarbonate concentration.

If the Pco_2 is 40 mm Hg, the relationship among the determinations is shown in eq. (1) and (2) for arterial and venous blood, respectively.

$$CT_a^{37°} = [CY^{20°} - 0.6] = [CC + 1.2] \geqq [CP + 1.2] \quad (1)$$

$$CT_v^{37°} = [CY^{20°} - 0.4] = [CC + 1.4] \geqq [CP + 1.4] \quad (2)$$

If the Pco_2 is above 40 mm Hg, the relationship is as in eq. (3);

$$CT > CC > CY \gtrless CP \quad (3)$$

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[1]Based upon and expanded from Weisberg [1,2] with permission of publishers, Williams and Wilkins [1] and ASCP [2].

[2]Figures in brackets indicate the literature references at the end of this paper.

Table 1. Comparison of various "CO_2" determinations.

Factors	CO_2 Content (total) CO_2 CT	CO_2 Capacity (total) CO_2 CY	CO_2 Combining capacity CO_2 CC	CO_2 Combining power CO_2 CP	CO_2 By auto-analyzer CO_2 A/A
Blood collection	anaerobic	anaerobic	anaerobic	aerobic	"aerobic"
Type of plasma used	true	true	separated	separated	separated
"CO_2" (or H_2CO_3) determined: Combined: $[HCO_3^-]$	yes	yes	yes	yes	yes
Free: $[H_2CO_3]$ + [diss CO_2]	yes	yes	no	no	no
Equilibration with CO_2 gas to 40 mm Hg[a]	none	20 °C	20 °C; 37 °C	20 °C; 37 °C	none[c]
Correction factors for free CO_2 (at 40 mm Hg)	none[b]	none	-1.8[a] -1.2[a]	-1.8[a] -1.2[a]	none
"Normal" values:					
Arterial (Pco_2 "40" mm Hg)	25.2 (23-27)[b]	25.8 (24-28)	24.0 (22-26)	CP $\leqq$ CC	(24-32)
Venous (Pco_2 "46" mm Hg)	27.0 (25-29)[b]	27.4 (25-29)	25.6 (24-28)	CP $\leqq$ CC	

[a] Respective proportionality constants ("solubility") of CO_2 at 20 °C and 37 °C are 0.046 and 0.03 ml/mm Hg.

[b] Result corrected to 37 °C.

[c] Original method (Skeggs) did equilibrate as for CO_2 CP.

and if the Pco_2 is below 40 mm Hg, the relationship is as in eq. (4).

$$CY > CC > CT \gtrless CP . \quad (4)$$

In 1883, Stadelmann [8] had formulated the concept of *"acidosis"* which resulted from *excess acid production* which could then result in *alkali deficit*. Naunyn [9] expounded on the use of the term acidosis due to *endogenous production of hydroxybutyric acid* in diabetes, which was then followed by the secondary alkali deficit or *hypoalkalinity* or *alkalipenia*. Acidosis was used to designate qualitative and/or quantitative changes of acid in the blood [10,11] but not the "reaction" of the blood [10].

From 1909, however, the consensus [12-15] seemed to revert to the position of Walter and of Stadelmann, the term *"acidosis"* being used to indicate *any type of acid* which could alter the acid-base balance, resulting in a lowering of the *alkaline reserve*. If the respiratory mechanism could decrease the carbonic acid concentration to maintain the "normal" bicarbonate:carbonic acid ratio, it was called *compensated acidosis*, but if the carbonic acid could not be reduced sufficiently, with an accompanying increase of hydrogen ion (pH decreased), Hasselbalch and Gammeltoft [16] called it *uncompensated acidosis*.

In 1931, Peters and Van Slyke [17] used the terms *1° alkali deficit* and *1° alkali excess* to denote changes in the blood. These can be traced back to 1877 (Walter [3]), and to 1883 (Stadelmann [8]). Changes in body content were called *"base" deficit* and *"base" excess* [17]. In 1947 (first edition), Davenport [18] modified the terms to describe blood changes as *extra fixed acid (or acid excess)* and *extra fixed base (or base excess)*. Singer and Hastings [19,20] used combinations of these terms--*fixed acid excess* (or *base deficit*) and *fixed acid deficit* (or *base excess*); Roos and Thomas [21] also use the terms fixed acid excess and fixed acid deficit for metabolic acidosis and metabolic alkalosis, respectively.

In 1948, Singer and Hastings [19,20] introduced the determination of *buffer base* which describes the buffer system of *whole blood*. It is the cation equivalent to the sum of *buffer* (or "labile") *anions* ("base")--bicarbonate, proteinate, and phosphate in the plasma, and the oxyhemoglobin and other erythrocyte buffer anions (*e.g.*, phosphate) [22,23]. The term "buffer base" was used by Stadie, *et al.* [23a] in 1925 to represent the bicarbonate and protein concentration.

The "non-bicarbonate" buffer base was designated as $[Buf^-]$; the *non-buffer* (or "fixed") *anions* consist of chloride, sulfate, and the organic acids. The pH and total carbon dioxide content are determined in whole blood, which is corrected for the hematocrit or hemoglobin content, and the partial pressure of carbon dioxide and "buffer base" calculated from a nomogram [20] or diagram [19].

The normal buffer base of *whole blood* of normal hematocrit is 49 mEq per liter (range 46 to 52) and, for *plasma*, 42 mEq per liter (range 39 to 45). The difference is due to the much higher buffer (oxyhemoglobin) content in the erythrocytes. Note the similarity of the definition of alkaline reserve of plasma by Cummer [4] and the buffer base of plasma (omitting hemoglobin factor for whole blood buffer base). The buffer base is decreased in metabolic acidosis and increased in metabolic alkalosis. Recently [24], buffer base has been equated with *total bicarbonate*.

Buffer base, originally represented as B_B+, was modified by Elkinton and Danowski [25] to BB to signify the *body buffers* which were equal to the total *buffer cations* (value is equivalent to the *buffer anions*, also termed *Buf^-*). The buffer base was the difference between the fixed (total) cations and the "fixed" anions, necessary to maintain electroneutrality [26].

The symbol for buffer base, as used by Owen, *et al.* [27], is B^-; Kintner and Gambino [28,29] used B for "base". Roos and Thomas [21] use BBB to represent *blood buffer base* and suggested the term *standard blood buffer base* or SBBB to designate the buffer base which would be obtained if mixed venous blood were brought to Pco_2 40 mm Hg *in vivo*. They utilize the Singer-Hastings nomogram [20] to derive the conventional BBB and the SBBB; both BBB and SBBB include the carbamino-CO_2 [21].

At a symposium on pH and blood gas measurement, Astrup [72] introduced the term Δ *acid-base* to represent the total amount of *surplus acid* or *surplus base* in one liter of blood at a given concentration of *standard bicarbonate* [50,51] (*vide infra*). One could calculate the amount of sodium bicarbonate or ammonium chloride needed to correct a surplus acid or base, respectively, by multiplying the Δ acid-base per liter by the body weight in kilograms times 0.3 (the extracellular space) [31,72].

In 1960, Astrup and his colleagues [30-32] changed acid excess and base excess as descriptive terms for the nonrespiratory factors to *base excess* ±. Base excess is defined as the deviation of the buffer base (ΔBB) from the "normal" buffer base. Instead of the actual titration of the blood to pH 7.40 at 37 °C at a Pco_2 of 40 mm Hg, it is much easier to calculate BE [33] or to derive it from the curve nomogram [34], alignment nomogram [35], or slide rules [36,37].

To avoid the conceptual ambiguity of "negative base excess" Lyons and Moore [38] use *buffer base deviation*; Kintner and Gambino [28,29] use *base deviation*. Suero and Woolf [39] use the term *nonrespiratory acid excess*.

Davenport [18] uses the terms base excess (as previously) and base deficit (for his previous acid excess) for blood changes; these were the terms used by Peters and Van Slyke [17] in 1931 for changes in body content. Davenport [18] utilized the bicarbonate-pH diagram for graphic representation of his terms extra fixed acid (or acid excess or base deficit)--point below the buffer slope of carbon dioxide--and extra fixed base (or base excess)--point above the line. I have used similar graphic representations [40] on a modified CO_2 content-pH diagram first described by Cullen and Jonas [41] and Hastings *et al.* [42]. The descriptive term for the (metabolic) acid-base alteration is *delta CO_2 content* (ΔCT), eq. (5).

$$\Delta \text{ CT} = \text{Actual } CO_2 \text{ CT} - \text{Theoretical } CO_2 \text{ CT} \tag{5}$$

The theoretical CO_2 content can be calculated for arterial and venous blood, utilizing a buffer slope or molar buffer value of 28, from the intercept of actual pH with the CO_2 buffer slope or from eqs. (6) and (7).

$$\text{Theoretical } CO_2 \text{ CT}_a = 25 + 28\ (7.40 - \text{pH}) \tag{6}$$

$$\text{Theoretical } CO_2 \text{ CT}_v = 27 + 28\ (7.37 - \text{pH}) \tag{7}$$

The values for arterial and venous theoretical CO_2 content also may be obtained from the table on the Weisberg TRI-SLIDE™ calculator for the Henderson-Hasselbalch equation. A "negative" delta content (ΔCT) signifies a bicarbonate (combined CO_2) deficit or metabolic acidosis and is equivalent to the "base excess negative" or "base deficit" whereas a positive ΔCT signifies bicarbonate (combined CO_2) excess or metabolic alkalosis and is equivalent to the "base excess positive" or "base excess". Changes in the bicarbonate concentration may be substituted for the CO_2 content changes but only when the Pco_2 value is normal.

Since the non-bicarbonate buffers [Buf^-] are not directly measurable, Filley [43] utilized the *corrected* $\Delta[HCO_3^-]$ as the base excess. However, the *actual* $\Delta[HCO_3^-]$--the difference between the actual plasma bicarbonate and 24 mEq per liter--seldom differs by more than 3 mEq per liter from the *"base excess"* [43]. Collier *et al.* [44] have applied the concept of base excess to the extracellular fluid (BE_{ECF}) rather than the blood. It can be calculated from their bicarbonate-pH diagram (with buffer line of extracellular fluid rather than that of plasma) or from their formula;

$$BE_{ECF} = [HCO_3^-]_p + \beta_{ECF}\ (\text{pH} - 7.4) - 24 \tag{8}$$

in which $[HCO_3^-]_p$ is the actual plasma bicarbonate and β_{ECF} is the *in vivo* apparent buffer value (approximately 12). One can transform this concept to that of "ΔCT" by altering eq. (5) to eq. (9).

$$ECF_{\Delta CT} = \text{Actual } CO_2 \text{ Content} - \text{Theoretical } ECF_{CO_2\ CT} \tag{9}$$

Similarly, eqs. (6) and (7) are transformed to eqs. (10) and (11).

$$\text{Theoretical } ECF_{CO_2\ CT_a} = 25 + 12\ (7.40 - pH) \tag{10}$$

$$\text{Theoretical } ECF_{CO_2\ CT_v} = 27 + 12\ (7.37 - pH) \tag{11}$$

Use of eqs. (9), (10), and (11) is much easier than the necessary plotting on diagrams and finding the vertical displacement, etc.; special slide rules [37,45,46] are easier to use than the mathematical calculations with formulas.

In 1930, Henderson, *et al.* [47] introduced the term, T_{40}, to represent the millimoles of *total carbonic acid* per liter of blood when the Pco_2 equals 40 mm Hg. The carbon dioxide content had also been standardized to a pH of 7.40. The formula of Peters and Van Slyke [17] for the *standardized carbon dioxide content* is

$$[CO_2CT]_{pH_{7.40}} = [CO_2CT] + (pH - 7.40)\ (8.2 + 2.6\ Hb) - 0.36\ HbO_2 \tag{12}$$

in which the carbon dioxide content, hemoglobin (as oxygen capacity), and oxygen content are expressed as millimoles per liter. Peters and Van Slyke [17] also had a formula for the *standardized bicarbonate*, adjusted to pH 7.40.

$$[HCO_3^-]_{pH_{7.40}} = [HCO_3^-] + (pH - 7.40)\ (8.2 + 2.3\ Hb) \tag{13}$$

The hemoglobin was expressed in terms of millimoles per liter which can be obtained by dividing grams percent by 1.67. This equation can be rewritten in terms of grams percent hemoglobin.

$$[HCO_3^-]_{pH_{7.40}} = [HCO_3^-] + (pH - 7.40)\ (8.2 + [1.37 \times g\%\ Hb]) \tag{14}$$

It was assumed that the total plasma protein concentration was constant at 7 g per 100 ml. In 1952, Bunker *et al.* [48] reported a modified formula for the *corrected bicarbonate*.

$$[HCO_3^-]_{pH_{7.40}} = [HCO_3^-] + (pH - 7.40)\ (8.6 + [1.4 \times g\%\ Hb]) \tag{15}$$

Eichenholz, *et al.* [49] determined the standardized bicarbonate using the intersection at pH 7.40 of a line parallel to the normal carbon dioxide absorption slope.

In 1957, Astrup and his associates [50,51] introduced the term *standard bicarbonate*, which is defined as the concentration of *bicarbonate* in plasma separated from the cells with the hemoglobin completely oxygenated at a Pco_2 of 40 mm Hg and at a temperature of 38 °C. They use the standard bicarbonate to characterize nonrespiratory ("metabolic") disturbances because the carbon dioxide content will vary with the Pco_2 and Po_2 present in the blood sample. The standard bicarbonate is approximately 1.2 mmol per liter less than the carbon dioxide *capacity* determined at a Pco_2 of 40 mm Hg but on arterial ("completely" oxygenated) blood at 38 °C (instead of the usual procedure utilizing venous blood at 20 °C). It is not as useful as changes in buffer base or of base excess [30]. The original [52] and modified [34] Siggaard-Andersen curve nomogram yields values for standard bicarbonate, etc. In 1967, however, the *standard* bicarbonate on the nomogram (distributed by Radiometer A/S) was changed to *plasma* bicarbonate (at Pco_2 40 mm Hg) with the supporting descriptive statement that this "plasma bicarbonate" is the standard bicarbonate.

Armstrong, *et al.* [53] prefer T_{40} *bicarbonate* (named after L. J. Henderson [47] who based many of his studies on blood at a CO_2 *tension* (Pco_2) of 40 mm Hg)--also equilibrated to Pco_2 40 mm Hg, but as if it occurred in the subject (*in vivo*) rather than after the sample is removed (*in vitro*). The T_{40} bicarbonate differs from the standard bicarbonate by the amount of *dilution* by the *interstitial fluid* of the bicarbonate generated during hypercapnia. As explained above, the standard bicarbonate can be described as a "modification" of the carbon dioxide capacity; similarly the T_{40} bicarbonate is similar to the carbon dioxide *combining capacity*, the bicarbonate concentration at a Pco_2 of 40 mm Hg (determined on separated plasma without the hemoglobin buffering capacity). It can be calculated from the Singer-Hastings nomogram [20] (using an "effective" (extracellular) hematocrit of 0.09) or the Astrup log Pco_2/pH diagram [30,52] (using an "effective" hemoglobin of 3 g per 100 ml); such calculations utilize a "dilution factor" of five to obtain the effective hematocrit or hemoglobin (based on ratio of blood volume to extracellular fluid and ratio of buffering capacity of blood and interstitial fluid). In addition, the Singer-Hastings nomogram and the Astrup diagram have been modified [44], allowing direct estimation of the T_{40} bicarbonate without hematocrit or hemoglobin values, respectively. The T_{40} bicarbonate can be estimated mentally if the Pco_2 is known. For "hypercarbia", eq. (16) is used [53], whereas for "hypocarbia", eq. (17) is used [54].

$$[HCO_3^-]_{\,in\ vivo} = [HCO_3^-]_{\,in\ vitro} - \frac{Pco_2 - 40}{15} \tag{16}$$

$$[HCO_3^-]_{\,in\ vivo} = [HCO_3^-]_{\,in\ vitro} - \frac{Pco_2 - 40}{10} \tag{17}$$

The *in vivo* buffer curve for carbon dioxide (expressed as $[HCO_3^-]$) is curvilinear rather than rectilinear [18,55]. Davenport [18] describes the relationship of bicarbonate for specific values of Pco_2 as eq. (18).

$$[HCO_3^-] = 31.39 \times \frac{Pco_2}{Pco_2 + 12.95} \tag{18}$$

The same curvilinear relationship holds for the carbon dioxide titration curve when expressed as carbon dioxide content. It is best therefore to estimate ΔCT or base excess/deficit from the *in vivo* buffer data when the pH is below 7.40 ("acidosis") and from the *in vitro* buffer data when the pH is above 7.40 ("alkalosis").

In similar fashion, Severinghaus and Bradley [56] state that the blood *in vivo* behaves as if the hemoglobin concentration were one-third (*e.g.*, 5 g per 100 ml) of the actual value since the extracellular fluid is about twice the blood volume and the bicarbonate, generated from carbon dioxide, diffuses from the blood into the extracellular fluid (*sic*). With the Siggaard-Andersen alignment nomogram [35] they use the one-third hemoglobin value on the

original line as a fulcrum to connect with Pco_2 40 mm Hg (rather than the "original" base excess with Pco_2 40) and thus derive the *in vivo* standard (plasma) bicarbonate and base excess (latter read at the actual hemoglobin concentration).

An *in vivo* carbon dioxide titration curve, based on a buffer capacity of about 5 g per 100 ml hemoglobin was reported by Siggaard-Andersen in 1967 [57] and expanded upon in 1971 [58], giving values for base excess and base deficit, and for bicarbonate at Pco_2 40 mm Hg.

Roos and Thomas [21] discuss the *"error"* of the conventional standard bicarbonate (SB *in vitro* minus SB *in vivo*) which should be subtracted from the *in vitro* value to obtain the estimated *in vivo* value of standard bicarbonate; the "error" varies with the pH, hematocrit, and Pco_2.

Owen *et al.* [27] proposed the term *appropriate bicarbonate*, that bicarbonate appropriate to the *actual* Pco_2 present in the patient. The difference between the appropriate and the actual (observed) bicarbonate values is a measure of the change in plasma buffers.

$$\Delta \text{ Plasma buffers} = [HCO_3^-]_{approp} - [HCO_3^-]_{obs} . \qquad (19)$$

In 1916, Hasselbalch [11] saturated blood with carbon dioxide at 37 °C under 40 mm tension and determined the *"reduced hydrogen ion concentration"* (or *reduced* pH or *nonrespiratory* pH). Under such conditions the carbonic acid concentration is fixed and the hydrogen ion concentration must vary inversely as the concentration of (sodium) bicarbonate; thus the "reduced hydrogen ion concentration" is a measure of the blood *bicarbonate* [14,14a].

In 1959, Peirce and his colleagues [59,60] determined the metabolic and respiratory pH factors by a "double pH" method--measuring the actual pH and the *eucapnic pH* (blood equilibrated to Pco_2 40 mm Hg). If the actual and eucapnic pH values were the "same", a pure "metabolic" imbalance could be present; a low pH indicated metabolic acidosis and high pH indicated metabolic alkalosis. If the eucapnic (but not the actual) pH equalled "7.40", a pure "respiratory" imbalance was present. If the actual pH was lower than the eucapnic pH, respiratory acidosis was present; conversely, if the actual pH was higher than the eucapnic pH, respiratory alkalosis was present.

In 1965, Whitehead [61,62] described changes in acid-base balance in terms of *hydrogen ion units*, expressed as nanoequivalents (or nanomoles) per liter. The pH values must be converted into hydrogen ion concentration; the Astrup diagram is used to determine the hydrogen ion concentration at Pco_2 40 mm Hg (with hemoglobin fully saturated). The total change is the sum of the changes in respiratory and nonrespiratory factors, $\Delta[H^+] = R + NR$.

The complete equation is,

$$([H^+]_{tot} - 40) = ([H^+]_{tot} - [H^+]_{Pco_2\ 40}) + ([H^+]_{Pco_2\ 40} - 40) \qquad (20)$$

in which $[H^+]_{tot}$ is the actual hydrogen ion concentration for the determined pH at the actual Pco_2 in the patient and $[H^+]$ Pco_2 40 is the hydrogen ion concentration for the pH at Pco_2 of 40 mm Hg. Each term has a "normal" range of ± 4 nmol; a negative sum means a deficit of hydrogen ions (alkalosis) and a positive sum, an excess of hydrogen ions or acidosis.

Kintner [63,64] describes acid-base changes in terms of A and B, respectively, the deviation of Pco_2 ("acid") and carbon dioxide content ("base") from their respective normal values. Subsequently, the "base" portion was equated with changes in buffer base (base excess and base deficit) and the Siggaard-Andersen alignment nomogram was used to determine the A and B factors; in addition, the term delta $[H^+]$ was used to quantitate the acid-base imbalance [28,29].

The *molar buffer value* was used by Van Slyke to describe buffering capacity (or buffer slope [43])--the number of moles of acid needed to decrease pH by one unit in one liter of a molar solution. In 1965, Woodbury [65] introduced the term *Van Slyke* or *slyke* (abbreviated sl) for the molar buffer value, defined as the *ratio* of the change in base [65] or bicarbonate [43] to the change in pH as the measure of "buffer capacity" of blood due to the presence of hemoglobin and plasma proteins. Hemoglobin has a buffer value of 3 sl/mmol and plasma protein has a value of 0.1 sl/g. Instead of converting hemoglobin as grams per deciliter to millimoles per liter by dividing g/dl by 1.67 or multiplying g/dl by 0.621, the entire relationship can be described in eq. (21),

$$sl/l = (1.86 \times Hb\ g/dl) + [TP\ g/dl \times (1 - Hct)] \qquad (21)$$

in which the Hb is hemoglobin, TP total protein, and Hct hematocrit.

Substitution of "average" values of 14 g/dl and 6 g/dl for hemoglobin and total protein, respectively, with a hematocrit of 0.45 gives 29.3 sl/l of blood. Filley [43] gives 29 sl/l as a normal value for blood of pH 7.3 to 7.5. For *"in vivo"* conditions as exemplified in a 70 kg individual with a blood volume of 7.1 percent of body weight ("average" of 7.6 percent for average male and 6.6 percent for average female), there are 146.5 sl in the 5 l of blood. The "interstitial fluid" volume in both sexes is about 12 percent of body weight and contains an average of 0.66 g/dl protein (with a hematocrit of "zero"); therefore 0.66 g/dl x 10 x 0.1 x 8.4 l yields 5.5 sl. The grand total of 152 sl divided by the 13.4 l of extracellular fluid yields a concentration of about 11.3 sl/l. Factors of 28 sl and 12 sl are utilized in eqs. (6) and (7), and (10) and (11), respectively.

Data for Pco_2, $[HCO_3^-]$, and $[H^+]$ changes have also been presented as *index values* (sample value/median normal value), permitting calculation of the compensation (and buffering) present as a percentage of the (calculated) "uncompensated" increase of hydrogen ion [66].

Table 2 summarizes some schemes for determining and describing acid-base alterations of the blood. The "old" system consisted only of measuring the alkaline reserve (carbon dioxide content), a decreased value establishing a diagnosis of acidosis [4]; the possibility that alkalosis could exist was denied [13].

There are no significant differences among the scheme that I use, or Siggaard-Andersen's [33] or the report (not recommendations, as originally intended) from the New York Academy of Sciences [67]. It was agreed that the preferential order for describing an acid-base alteration is to (a) use the specific numerical data for the test result, (b) describe the test values as "high, low or normal", or (c) use the general "descriptive" terms.

The *ad hoc* committee [67] suggested that acidemia and alkalemia be used to describe alterations of blood pH and to utilize acidosis and alkalosis to describe the overall physiological process or condition which tends to cause a deviation in pH without being dependent upon deviation of the pH *per se*.

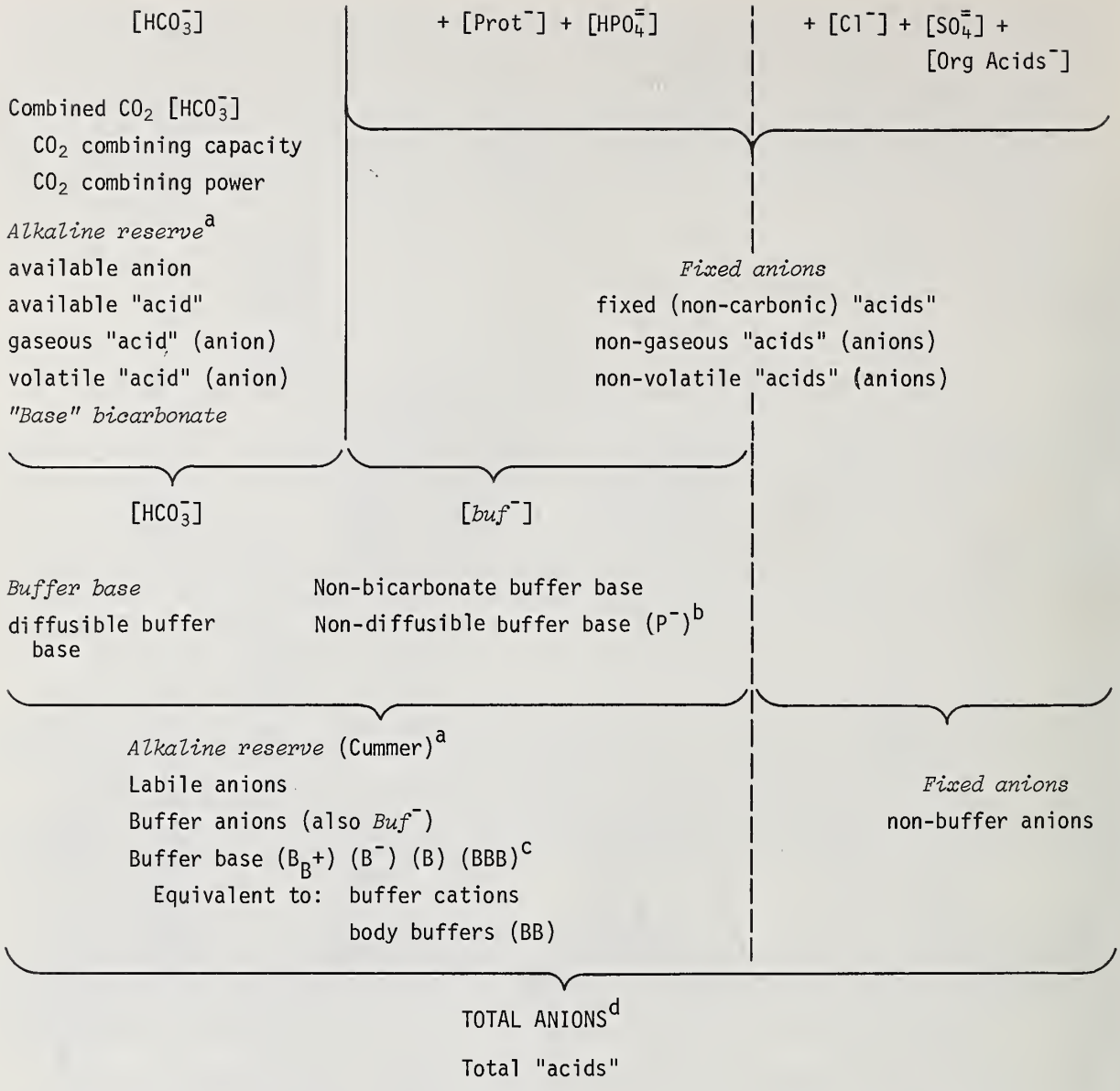
My criticism of acidemia-alkalemia is that these terms refer to blood and leave the pH of the interstitial fluid (in equilibrium with the intravascular fluid) in limbo. When pH changes of the intracellular compartment are reported (primarily research), it is so designated since the normal values differ from that of the "extracellular" fluid. These comments are even more relevant today with the emphasis on the *in vivo* titration curve, etc. [44,56-58]. A possible compromise may be to use *eupHemia* and *eupHuria* to represent a normal pH of blood and urine, respectively, since these terms "are compact, easily spelled, and easily pronounced" [68]. In a similar vein one could use the terms *hypopHemia* and *hyperpHemia* to designate decreased pH (acidosis and/or acidemia) and increased pH (alkalosis and/or alkalemia), respectively.

All agree that the respiratory changes are determined by measuring Pco_2. I have designated the test as Pco_2 or $[H_2CO_3]$; the latter (used by Peters and Van Slyke) is equal to 0.03 x Pco_2 and emphasizes the carbonic acid term, similar to that of the Henderson-Hasselbalch equation, avoiding confusion with the various "CO_2" determinations. The descrip-

Table 2. Schemes for determining and describing acid-base alterations of blood.

"Old"		Weisberg		Siggaard-Andersen [33]		Tentative report of NYAS conference [67]	
Determination	Descriptive term	Determination	Descriptive term	Determination	Descriptive term	Determination	Descriptive term
		↑	alkalosis	↑	(total) baseosis	↑	"alkalemia"
?pH	?	pH		pH		pH	
		↓	acidosis	↓	(total) acidosis	↓	"acidemia"
	"respiratory acidosis"	↑	carbonic acid (free CO_2) excess	↑	respiratory acidosis	↑	hypercapnia
?		Pco_2 or $[H_2CO_3]$		Pco_2		Pco_2	
	"respiratory alkalosis"	↓	carbonic acid (free CO_2) deficit	↓	respiratory baseosis	↓	hypocapnia
?	"metabolic alkalosis"	+	bicarbonate (combined CO_2) excess	+	nonrespiratory baseosis	↑ (or +)	"hyperbasemia"
Alkaline reserve (CO_2 content)		ΔCO_2 content[a]		base excess		bicarbonate buffer base std bicarb base excess	
↓	"metabolic acidosis"	-	bicarbonate (combined CO_2) deficit	-	nonrespiratory acidosis	↓ (or -)	"hypobasemia"

[a] ΔCO_2 Content = Actual CO_2 Content - Theoretical CO_2 Content



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[a] Additional "definitions" of *alkaline reserve*:
Total CO_2 content: combined CO_2 $[HCO_3^-]$ + free CO_2 $[H_2CO_3]$
CO_2 capacity: (combined CO_2 + free CO_2) at Pco_2 40 mm Hg and 20 °C
Portion of $[Na^+]$: "*base*" *bicarbonate* or bicarbonate-bound "base" or available alkali or available "base" or available cation.
Total cations or total "base" or fixed cations or fixed "base."

[b] Δ Non-diffusible buffer base also includes hemoglobin proteins.

[c] *Buffer base* of plasma also defined as bicarbonate plus protein. *Buffer base* of whole blood also includes buffer activity of hemoglobin, etc. of erythrocytes.

[d] Total anions minus those anions which have been determined equals RESIDUAL ANIONS or Residual "acids."

Figure 1. Terms applied to various fraction of total plasma anions modified from Weisberg [2]; duplicated terms in italics.

tive terms of the committee for respiratory alterations do not meet with my support. For conformity with acidemia and alkalemia, they should use hyper- and hypo-capnemia. There is easy confusion between the words -capnia (Greek root *kapnos* meaning smoke or vapor) and -capnea (Greek root *pnoe* meaning breathing). Some authors have used the terms hyper- and hypo-carbia (Latin root *carbo* meaning coal), which are "barbarisms"--combinations of Greek prefixes and Latin nouns [69].

It is obvious that the major disagreements are in relation to the diagnosis of the "metabolic" alterations--especially which test to use. Utilizing the Brønsted concept, all anions are *conjugate bases* and could fit into the descriptive terms (hyper- or hypo-basemia) of the committee report, but only the bicarbonate anion and organic acid anions have a primary effect on the hydrogen ion concentration. Bicarbonate is considered to be part of the labile "buffer" anions (equivalent to buffer base) whereas the organic acids are part of the fixed "non-buffer" anions.

Although not intended to confuse the reader, figure 1 shows the interrelationships of some of the various terms applied to different components of the total anions; some duplications are self-evident. Though *all* the anions are "conjugate bases" and can act as a base by accepting a proton, the "buffer" anions are the major buffers since their pK' values are closer to the pH of blood (the pK' values of the "fixed" anion systems are lower).

Figure 2 is a simple flow chart for the differential diagnosis of acid-balance imbalance. Of potential thirteen diagnoses, seven can be distinguished by use of pH and Pco_2. To distinguish between a pure respiratory or a mixed respiratory and metabolic imbalance, the actual bicarbonate concentration or CO_2 content is utilized; and to distinguish between an acute or chronic respiratory imbalance, the base excess/deficit or delta CO_2 content is necessary.

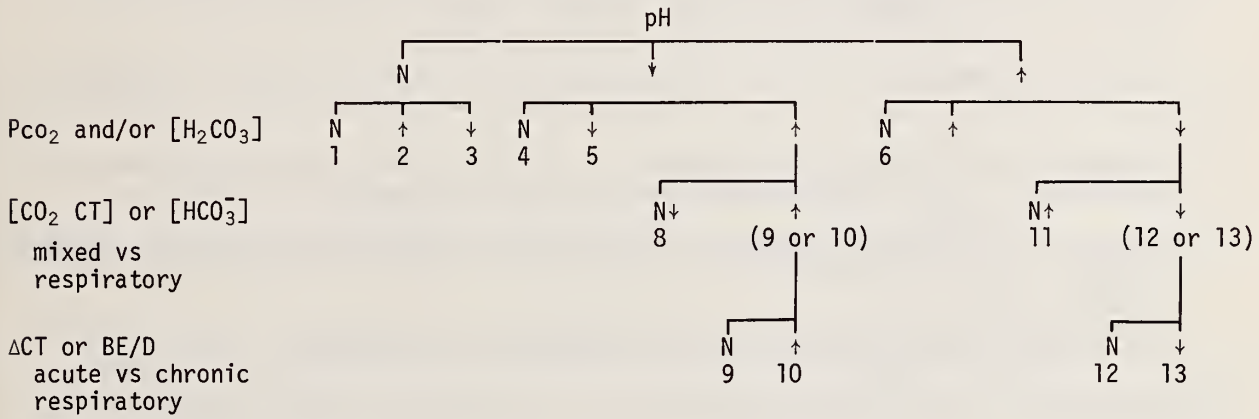

Figure 2. Flow chart for differential diagnosis of acid-base imbalance.
Key to numbers in chart:

1) normal acid-base
2) resp acid, comp (met alk, comp)
3) resp alk, comp (met acid, comp)
4) met acid, acute
5) met acid, partial comp
6) met alk, acute
7) met alk, partial comp
8) mixed resp acid and met acid
9) resp acid, acute
10) resp acid, chronic or partial comp
11) mixed resp alk and met alk
12) resp alk, acute
13) resp alk, chronic or partial comp

It is ironic that we have come full circle in the course of a century. As stated by Filley [43], "Ambiguity in the meaning of words, a major curse of specialization, arises

both because words often outlive the concepts they originally stood for and because the same word is used by different experts to mean different things, *i.e.*, abused." Additional commentaries are by two poets--ex-Senator Eugene McCarthy [70] said, "If the language is debased or misused, if the meaning of words is obscured, the basis for common judgment is undermined, if not destroyed"; and W. H. Auden [71] said "... there is only one political duty, and that is to defend one's language from corruption. When it's corrupted, people lose faith in what they hear, and this leads to violence."

If one can communicate without misunderstanding, it does not make much difference which "terms" are used! One cannot, however, take the position described by Lewis Carroll in *Through the Looking Glass*--"When I use a word, Humpty Dumpty said, in a rather scornful tone, it means what I choose it to mean. Neither more nor less."

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TRI-SLIDE™ CALCULATOR FOR HENDERSON-HASSELBALCH EQUATION AND CO_2RREC°t-O_2-SLIDE™ FOR TEMPERATURE CORRECTIONS OF pH, Pco_2, AND Po_2

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The general form of the Henderson-Hasselbalch equation

$$pH = pKa + \log \frac{[\text{Proton Acceptor}]}{[\text{Proton Donor}]} \quad (1)$$

may be rewritten for the bicarbonate:carbonic acid system with the usual *apparent* pK' of 6.10 (in whole blood or plasma at 37 °C body temperature) as eq (2).

$$pH = 6.1 + \log \frac{[HCO_3^-]}{[H_2CO_3]} \quad (2)$$

The total carbon dioxide content (CO_2 CT) is *essentially* the sum of the bicarbonate (*combined* CO_2) and carbonic acid (*free* CO_2); the latter is *proportional* to the partial pressure of carbon dioxide (Pco_2 symbol as used by respiratory physiologists) and *includes* the *dissolved* (free) carbon dioxide.

$$[CO_2\ CT] = [HCO_3^-] + [H_2CO_3] \quad (3)$$

$$[HCO_3^-] = [CO_2\ CT] - [H_2CO_3] \quad (4)$$

$$[H_2CO_3] = \alpha\, Pco_2 = 0.03\ Pco_2 \quad (5)$$

Since $[HCO_3^-]$ and $[H_2CO_3]$ are not directly determined, eq (4) and (5) are substituted into eq (2) giving rise to eq (6).

$$pH = 6.1 + \log \frac{[CO_2\ CT] - 0.03\ Pco_2}{0.03\ Pco_2} \quad (6)$$

1. TRI-SLIDE

If any two of the three unknown quantities in eq (6) are known, the third can be calculated [1-3][1]. The TRI-SLIDE Calculator (fig. 1) (first designed in 1963 with revisions in 1965, 1969, and 1971) has been redesigned; it reduces all calculations to one setting of the special slide rule [4-6].

The calculator has a table summarizing the three possible settings. If the pH and Pco_2 and/or carbonic acid values are known, setting the Pco_2 value on the left hand movable scale (or the $[H_2CO_3]$ on right hand movable scale) opposite the pH value on center section allows one to read, opposite the red arrow (at 7.61 on pH scale), the $[CO_2\ CT]$ on the left hand

[1] Figures in brackets indicate the literature references at the end of this paper.

scale. If the pH and [CO_2 CT] values are known, the Pco_2 and/or [H_2CO_3] values are obtained by setting the [CO_2 CT] on left scale opposite red arrow and reading the Pco_2 and/or [H_2CO_3] opposite the pH. Finally, the pH can be determined if the [CO_2 CT] and Pco_2 and/or [H_2CO_3] values are known; the [CO_2 CT] is set opposite the red arrow and the pH read opposite the Pco_2 and/or [H_2CO_3].

Normal average values (and ranges) for adult males and females for arterial and venous blood are given in a table on the calculator (fig. 1); in addition, "normal" ranges are bracketed in red and blue (for arterial and venous blood, respectively) on the various slide

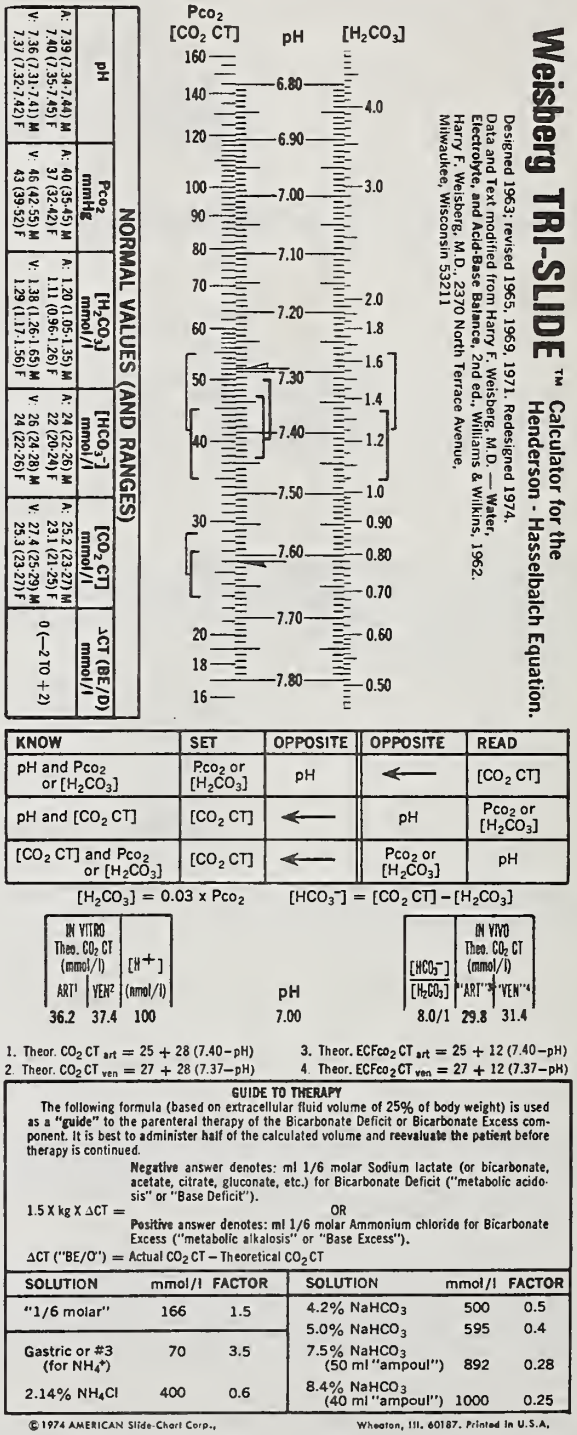

Figure 1. TRI-SLIDE calculator.

rule scales. The carbonic acid, bicarbonate, CO_2 content, and ΔCT (defined below) are expressed as millimoles per liter (mmol/l). The values for Pco_2 are given in terms of pressure (P) as millimeters of mercury (mmHg) and have been expressed as "torr" units; they also can be expressed (in keeping with the recommendation of the IUPAC and IFCC) in terms of pressure (p) as kilopascals (kPa).

One pascal (Pa) equals 1 newton (N) per square meter (N/m^2); 1 bar equals 100 000 N/m^2 or 100 kPa; 1 mbar equals 0.1 kPa or 0.75 mmHg whereas 1 mmHg (or 1 "torr") equals 1.333 22 mbar or 0.133 322 kPa; and 1 kPa equals 7.5 mmHg or 10 mbar. Equation (5) gave the concentration of carbonic acid utilizing the factor of 0.03 (at 37 °C) and the partial pressure of carbon dioxide (Pco_2) expressed in mmHg; for the "proposed" SI notation, eq (5) can be rewritten as

$$[H_2CO_3] = 0.23\ pCO_2 \tag{7}$$

in which the partial pressure of carbon dioxide (symbols pCO_2 or pco_2) is expressed in kPa. Table 1 compares the gas values in terms of mmHg and kPa.

The delta content (ΔCT) has the *same* connotation as Base Excess/Deficit.

$$\Delta CT \text{ (or "BE/D")} = \text{Actual } [CO_2 \text{ CT}] - \text{Theoretical } [CO_2 \text{ CT}] \tag{8}$$

Table 1. Comparison of "gas" values.

	PRESENT (mmHg) (mmHg = 7.5 x kPa)	"PROPOSED" (kPa) (kPa = 0.133 x mmHg)
Pco_2	A: 40 (35-45)M 37 (32-42)F V: 46 (42-55)M 43 (39-52)F	A: 5.3 (4.7-6.0)M 4.9 (4.3-5.6)F V: 6.1 (5.6-7.3)M 5.7 (5.2-6.9)F
Po_2	A: 95 (75-100) V: 40 (30-50)	A: 12.7 (10-13.3) V: 5.3 (4.0-6.7)
P_{50} (T_{50})	"26.6"	"3.5"

A *positive* delta content (ΔCT) signifies Bicarbonate or Combined CO_2 Excess (metabolic alkalosis) and is equivalent to Base Excess (BE) or "Base Excess Positive." A *negative* delta content (ΔCT) signifies Bicarbonate or Combined CO_2 Deficit (metabolic acidosis) and is equivalent to Base Deficit (BD) or "Base Excess Negative."

The "theoretical" [CO_2 CT] for arterial or venous blood (*in vitro* and *in vivo* or "extracellular") can be obtained from (a) the window on the calculator (which also gives the equivalent hydrogen ion concentration as nanomoles per liter (nmol/l) and the ratio of bicarbonate:carbonic acid for various pH values); (b) e.g.,

$$\text{Theoretical } CO_2\ CT_{art} = 25 + 28\ (7.40 - pH) \tag{9}$$

$$\text{Theoretical } CO_2\ CT_{ven} = 27 + 28\ (7.37 - pH) \tag{10}$$

$$\text{Theoretical ECF}_{CO_2\ CT_{art}} = 25 + 12\ (7.40 - pH) \tag{11}$$

$$\text{Theoretical ECF}_{CO_2\ CT_{ven}} = 27 + 12\ (7.37 - pH); \tag{12}$$

or (c) the Weisberg Acid-Base Balance Evaluation Diagram (fig. 2) from the intersection of the *actual pH* of the specimen and the *in vitro* or *in vivo respiratory buffer lines*.

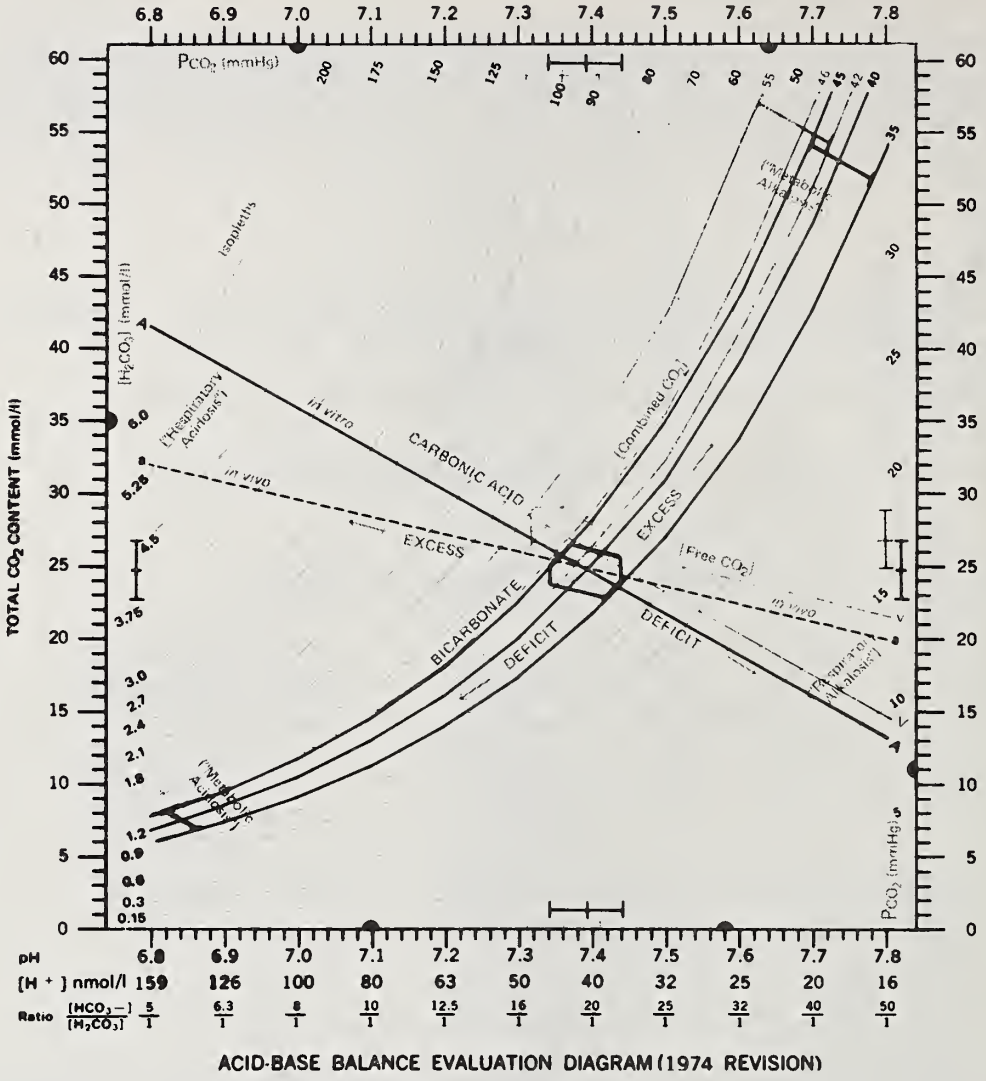

Figure 2. Acid-base balance evaluation diagram.

The buffering capacity--the number of moles of acid needed to decrease pH by one unit in one liter of a molar solution--was termed the *molar buffer values* by Van Slyke. Woodbury [7] introduced the term *Van Slyke* or *slyke* (abbreviated *sl*) for the molar buffer value, defined as the *ratio* of the change in base [7] or bicarbonate [8] to the change in pH as the measure of "buffer capacity" of blood due to the presence of hemoglobin and plasma proteins. Hemoglobin has a buffer value of 3 sl/mmol (or 1.86 sl per g/dl) and plasma protein has a value of 0.1 sl/g. The entire relationship can be described in eq (13),

$$sl/l = (1.86 \times Hb\ g/dl) + [TP\ g/dl \times (1 - Hct)] \qquad (13)$$

in which the Hb is hemoglobin, TP total protein, and Hct hematocrit.

Substitution of "average" values of 14 g/dl and 6 g/dl for hemoglobin and total protein, respectively, with a hematocrit of 0.45 gives 29.3 sl/l of blood. Filley [8] gives 29 sl/l

as a normal value for blood of pH 7.3 to 7.5. For *"in vivo"* conditions as exemplified in a 70 kg individual with a blood volume of 7.1 percent of body weight ("average" of 7.6 percent for average male and 6.6 percent for average female), there are 146.5 sl in the 5 l of blood. The "interstitial fluid" volume in both sexes is about 12 percent of body weight and contains an average of 0.66 g/dl protein (with a hematocrit of "zero"); therefore 0.66 g/dl x 10 x 0.1 x 8.4 l yields 5.5 sl. The grand total of 152 sl divided by the 13.4 l of extracellular fluid yields a concentration of about 11.3 sl/l. Factors of 28 sl and 12 sl are utilized in eqs (9) and (10), and (11) and (12), respectively, and for the theoretical CO_2 content values on the TRI-SLIDE table (fig. 1) and in the *in vitro* and *in vivo* buffer lines on the Weisberg Acid-Base Balance Evaluation Diagram (fig. 2).

The *in vivo* buffer curve for carbon dioxide (expressed as $[HCO_3^-]$) is curvilinear rather than rectilinear [9-11] and expressed as eq (14) by Davenport [9] to give the value for bicarbonate at specific values of Pco_2 (which fall along the buffer curve).

$$[HCO_3^-] = 31.39 \times \frac{Pco_2}{Pco_2 + 12.95} \qquad (14)$$

The same curvilinear relationship holds for the carbon dioxide titration curve when expressed as $[CO_2\ CT]$. It is *best* to *estimate* the ΔCT (or BE/D) from the *in vivo* buffer data when the pH is *below* "7.40" ("acidosis") and from the *in vitro* buffer data when the pH is *above* "7.40" ("alkalosis").

2. Diagnosis

Seven out of 13 possible conditions of acid-base imbalance can be diagnosed (fig. 3) from the pH and Pco_2 and/or $[H_2CO_3]$. The CO_2 content *or* the actual bicarbonate value is used to distinguish a "mixed" imbalance from respiratory conditions; and the ΔCT *or* base excess/deficit (BE/D) is needed to differentiate between acute and chronic (partial compensated) respiratory conditions. The ΔCT or BE/D is *also* used in calculating the amount and type of fluid therapy required.

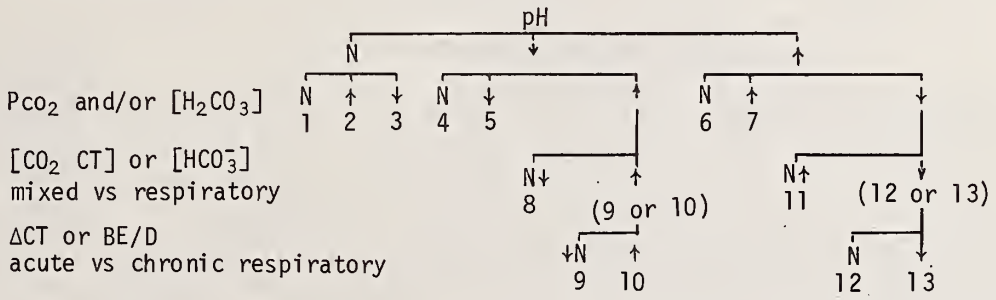

Figure 3. Flow chart for differential diagnosis of acid-base imbalance (see fig. 4).

Key to numbers in chart:

1) normal acid-base
2) resp acid, comp (met alk, comp)
3) resp alk, comp (met acid, comp)
4) met acid, acute
5) met acid, partial comp
6) met alk, acute
7) met alk, partial comp
8) mixed resp acid and met acid
9) resp acid, acute
10) resp acid, chronic or partial comp
11) mixed resp alk and met alk
12) resp alk, acute
13) resp alk, chronic or partial comp

The same diagnostic area numbers in figure 3 are used for diagnosis with the evaluation diagram as exemplified in figure 4. The "average line" of compensation is shown rather than overlapping "areas."

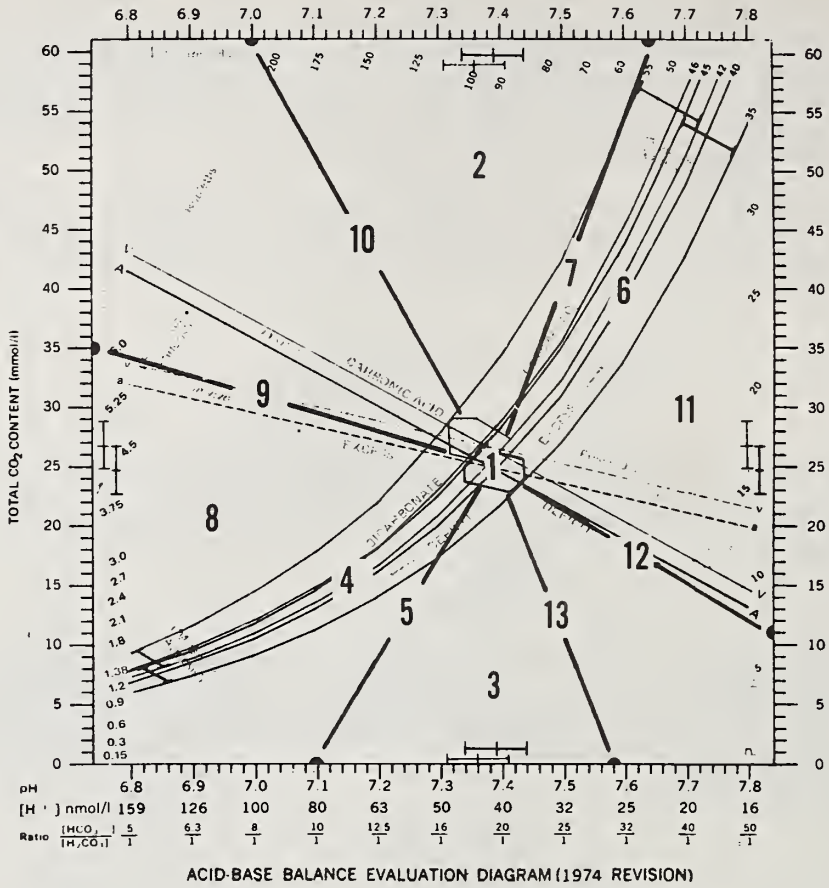

Figure 4. Acid-base diagram with lines for diagnosis and "confidence limits" (see fig. 3).

3. Therapy

The physician should utilize the laboratory data in the management of a patient with acid-base imbalance. Equation (15), based on an extracellular fluid volume of 25 percent of body weight, is used as a *"guide"* to the *parenteral therapy* of the Bicarbonate Deficit or Bicarbonate Excess component.

$$1.5 \times kg \times \Delta CT \text{ (or "BE/D")} = \begin{cases} \textit{Negative} \text{ answer denotes: } \textit{ml} \text{ 1/6 molar sodium lactate (or bicarbonate, acetate, citrate, gluconate, etc) for Bicarbonate Deficit (metabolic acidosis or "Base Deficit")} \\ \text{OR} \\ \textit{Positive} \text{ answer denotes: } \textit{ml} \text{ 1/6 molar ammonium chloride for Bicarbonate Excess (metabolic alkalosis or "Base Excess")} \end{cases} \quad (15)$$

The 1.5 "therapy factor" applies to *1/6 molar* solutions--about 166 mmol of cations and of anions (see table 2 for factors for other solutions)! It is best for the physician to *administer half* of the calculated volume and *reevaluate the patient* before therapy is continued [1,12].

Table 2. Factors for parenteral fluid therapy.

SOLUTION	mmol/l	FACTOR	SOLUTION	mmol/l	FACTOR
"1/6 molar"	166	1.5	4.2% $NaHCO_3$	500	0.5
			5.0% $NaHCO_3$	595	0.4
Gastric or #3 (for NH_4^+)	70	3.5	7.5% $NaHCO_3$ (50 ml "ampoul")	892	0.28
2.14% NH_4Cl	400	0.6	8.4% $NaHCO_3$ (40 ml "ampoul")	1000	0.25

4. CO_2rrec°t-O_2-Slide

The pH, Pco_2, and Po_2 values are usually determined at 37 °C with the instruments available in the clinical laboratory. This slide rule (fig. 5) is used to determine the respective values at 37 °C if they were determined at *other* temperatures, *e.g.*, water bath inaccurate or under special conditions. It is also used to determine the values "corrected" to the temperature of the patient.

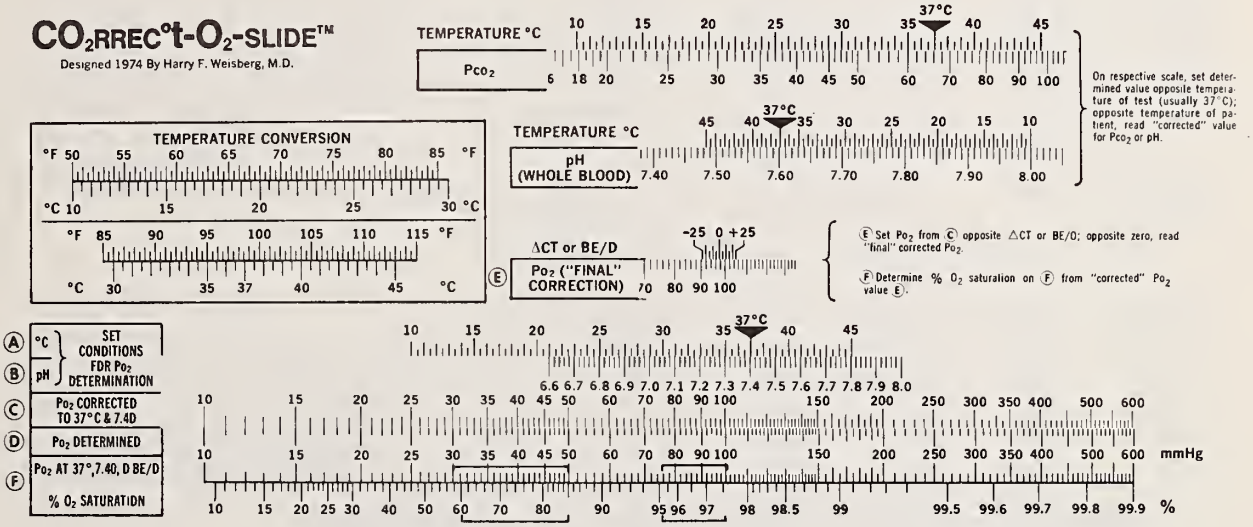

Figure 5. CO_2rrec°t-O_2-Slide.

It is imperative to correct pH, Pco_2, and Po_2 values to the patient's temperature rather than assuming 37 °C as for the thermostated water bath. Random patients were tested with an IVAC Model 811 electronic thermometer (°F) with the oral probe placed into the sublingual pocket for about 15 seconds. The accuracy of these thermometers is 0.15 °F in contrast to 0.5 °F for glass (mercury) thermometers. Figure 6 shows the range of temperatures found in 383 patients--94.5 to 101.7 °F (or 34.8 - 38.5 °C); the average temperature was 97.6 °F or 36.5 °C. The "discrepancy" will be greater with patients subjected to hypothermia or those with high fevers.

Equation (16) is utilized on the CO_2rrec°t-O_2-slide to calculate the pH of *whole blood* at the patient's temperature (pH_{o_t}) from the pH value as it was determined, usually at 37 °C (pH_{37°) [13].

$$WB\ pH_{o_t} = pH_{37^\circ} + 0.0147\ (37 - {}^\circ t)\ . \qquad (16)$$

Since plasma or serum [14] and cerebrospinal fluid contain less protein, the factors are different and the respective formulas are given in eqs (17) and (18).

$$P/S\ pH_{o_t} = pH_{37^o} + 0.012\ (37 - {}^\circ t) \tag{17}$$

$$CSF\ pH_{o_t} = pH_{37^o} + 0.003\ (37 - {}^\circ t) \tag{18}$$

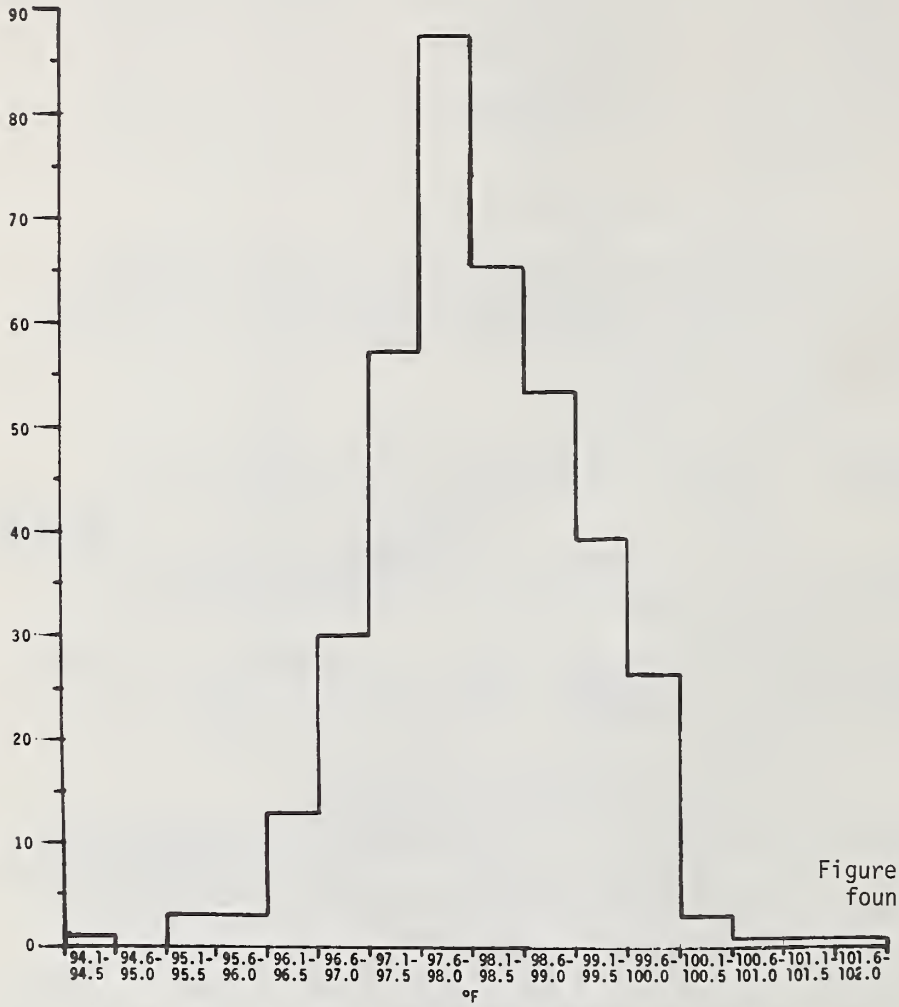

Figure 6. Range of temperatures found in 383 patients.

In addition, the actual Po_2 of the patient can be determined if the percentage oxygen saturation ("corrected") and the pH at the actual temperature of the patient are known. Finally, the CO_2rrec°t-O_2-slide allows one to correct the Po_2 to the temperature of the patient, and to correct Po_2 values to 37 °C, 7.40, and zero ΔCT (BE/D) for entrance into the oxyhemoglobin dissociation curve. The Po_2/% O_2 saturation scale (F) on the slide rule applies to "adult" hemoglobin A; the oxyhemoglobin dissociation curve of the *newborn* is different [15]! Other factors (2,3-DPG, type of hemoglobin, *etc.*) and various disease states can cause a shift of the position of the oxyhemoglobin dissociation curve.

The A (temperature) and B (pH) scales are used to set the conditions at which the test was done or to conditions desired for calculated results. The C scale shows Po_2 *corrected* to 37 °C and 7.40, whereas D scale shows *determined* Po_2; the E scale is used to correct Po_2 for existing changes in ΔCT or BE/D. The F scale is the "normalized" (37 °C, 7.40, and zero ΔCT or BE/D) oxyhemoglobin dissociation curve (Po_2/% O_2 saturation) up to 600 mmHg and 99.9 percent oxygen saturation.

Table 3 compares temperature corrections for various hypothetical conditions as calculated by different nomograms or slide rules.

Table 3. Comparison of corrections of pH, Pco_2, and Po_2 to temperature of patient.

I.	If patient's temperature is				
A. pH 7.40[a]	20°C	30°C	35°C	40°C	45°C
CO_2rrec°t-O_2-slide	7.65	7.50	7.43	7.36	7.28
Severinghaus Calculator [16]	7.67	7.51	7.43	7.36	7.29
Siggaard-Andersen Nomogram [17][b]	7.66	7.50	7.43	7.35	-
Kelman & Nunn Nomogram [18]	7.65	7.50	7.43	7.36	-
B. Pco_2 40 mmHg[a]					
CO_2rrec°t-O_2-slide	17.5	28.5	36.5	46.5	59
Severinghaus Calculator [16]	17.2	28.8	36.7	45.5	53.3
Siggaard-Andersen Nomogram [17][c]	18	29	36.5	45	-
Severinghaus Factors [19]	-	29.7	36.7	45.5	56.3
Kelman & Nunn Nomogram [18]	19.2	29.6	36.8	45.6	-
Greenburg & Moulder Nomogram [20]	18	29	36.5	45	56
Rattenborg [25]	19.5	30	37	46	57
C. Po_2 90 mmHg[a]					
CO_2rrec°t-O_2-slide	35	61	80	107	140
Severinghaus Calculator [16]	27.2	55	78	111.5	>150
Severinghaus-Astrup Nomogram [19,21,22][e]	34	60	80	105	140
Severinghaus Factors [19]	27.5	54.9	78.3	111.8	158
Severinghaus Factors [23]	1	53.2	79.5	105.8	132
Kelman & Nunn Nomogram [18][d]	36	61.2	81	103.5	-
Kelman & Nunn Factors [18]	-	60.8	80.7	105.9	-
Rattenborg [25]	27	55	79	-	-
Instrumentation Laboratory Nomogram [26]	-	59	79	105	140

II.	For Pco_2 values[a]				
If Patient at 34°C	30	40	50	60	70
CO_2rrec°t-O_2-slide	26	34.6	43.3	52	60.5
Severinghaus Calculator [16]	26.2	35	43.6	52.5	61
Siggaard-Andersen Nomogram [17][c]	27	35	43	51	59
Severinghaus Factors [19]	26.4	35.2	44	52.8	61.6
Kelman & Nunn Nomogram [18]	26.1	34.8	43.5	52.2	60.9
Greenburg & Moulder Nomogram [20]	26	34	44	52	61
Rattenborg [25]	26.5	35.5	44	53	61

III.	For pH values[a]					
A. If Patient at 34°C	7.00	7.10	7.20	7.30	7.50	7.60
CO_2rrec°t-O_2-slide	7.045	7.145	7.245	7.345	7.545	7.645
Severinghaus Calculator [16]	7.040	7.142	7.243	7.348	7.550	7.650
Siggaard-Andersen Nomogram [17][b]	7.045	7.140	7.245	7.340	7.540	7.640
Kelman & Nunn Nomogram [18]	7.045	7.145	7.245	7.345	7.545	7.645
B. If Patient at 34°C (Po_2 = 90 mmHg)						
CO_2rrec°t-O_2-slide	113	101	91	82	65	58
Severinghaus Calculator [16]	113	102	91	81.5	65.5	58.3
Severinghaus-Astrup Nomogram [19,21,22][e]	120	106	95	86	68	60
Kelman & Nunn Factors [18]	115	102.5	92	82.5	66	59
Instrumentation Laboratory Nomogram [26]	112	100	90	80	65	58

[a] As determined on laboratory instrumentation at 37°C.

[b] Modified from 38° to 37°C by substracting 0.02 pH.

[c] Modified from 38° to 37°C by adding 2 mmHg.

[d] Using 96% saturation line.

[e] Radiometer Chart No. 984-204.

By definition, the P_{50} (or T_{50}) value is the partial pressure of oxygen (corrected to 37 °C, pH 7.40, and BE/D (or ΔCT) of zero) corresponding to 50 percent oxygen saturation; normal value is about 26.6 mmHg or 3.5 kPa. The methods to obtain the P_{50} value are time-consuming. Canizaro, *et al.* [24] have developed a nomogram which allows *estimation* of the P_{50} value from a single *venous* blood sample on which the Po_2 (*corrected to patient's body temperature and pH*) and the percent oxygen saturation are determined. The estimated P_{50} values compare favorably (r = 0.92) with the actual P_{50} values if the oxygen saturation (venous blood) is between 25 and 75 percent.

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AIDS FOR EVALUATION OF ACID-BASE IMBALANCE--DIAGRAMS, NOMOGRAMS, AND SLIDE-RULES

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In 1962 I had accumulated 12 acid-base diagrams spanning the period, 1921-1959 (table 1). Since then I have been able to add 7 time charts (1917-1974), 17 nomograms (1923-1974) [45,46,162,166-180][1], 8 mathematical factors (1957-1967), and 12 slide rules (1958-1974) [181-192]. The original 12 diagrams have expanded to 39 for the period 1921-1959.

Table 1. Evolution of acid base diagrams. Reproduced with permission of the publisher, Williams and Wilkins [1].

Author	Year	Vertical coordinate (ordinate)		Horizontal coordinate (absicissa)		Other coordinates	
		Value	Log scale?	Value	Log scale?	Value	Log scale?
Van Slyke	1921	CT[a]		Pco_2		pH[b]	
Peters	1923	CT	Yes	Pco_2	Yes	pH	
Peters & Van Slyke	1931	HCO_3^-[c] or CT		pH			
Shock & Hastings	1934	HCO_3^-	Yes	pH		Pco_2[d]	Yes
Shock & Hastings	1935						
Davenport	1947	HCO_3^-		pH		Pco_2	Yes
Weisberg	1950						
Westerfeld	1953						
Weisberg	1956	CT		pH		Pco_2	Yes
Winters *et al.*	1958	CT	Yes	pH		Pco_2	Yes
Weisberg	1961	CT		$[H^+]$[e]		Pco_2	Yes
Singer	1951	BB[f]		Pco_2		pH and CT	Yes
Peirce *et al.*	1959	Pco_2		pH		BB	Yes
Brewin *et al.*	1955						
Astrup	1956	Pco_2	Yes	pH			
Astrup *et al.*	1959	Pco_2	Yes	pH		BB, BE,[g] and SB[h]	

[a]CT--carbon dioxide content; [b]pH--reciprocal of hydrogen ion concentration; [c]HCO_3--bicarbonate; [d]Pco_2--partial pressure of carbon dioxide; [e]$[H^+]$--hydrogen ion concentration; [f]BB--buffer base; [g]BE--base excess; and [h]SB--standard bicarbonate.

[1]Figures in brackets indicate literature references at the end of this paper.

Figure 1 illustrates the fecundity of the "sixties" for the production of acid-base aids. The earliest diagram I have been able to unearth was published in 1914 by Christiansen, Douglas and Haldane [3]. Table 2 is the latest accumulation of 105 diagrams (1914-1973) used in the evaluation of acid-base imbalance. The references listed in the 1° column are the originals I have been able to find, whereas those references in the 2° column are corrections, modifications, and variations based on the originals.

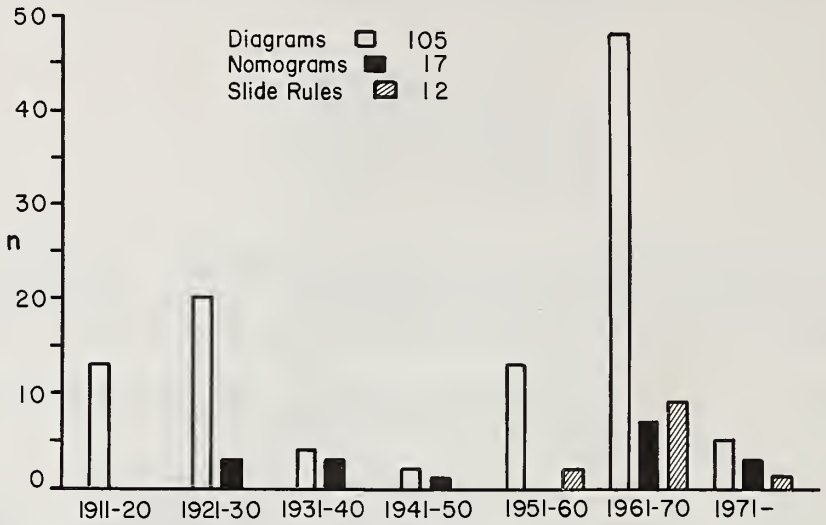

Figure 1. Histogram of acid-base aids. Modified from Weisberg [2].

Table 2. Comparison of 105 acid-base diagrams. Modified from Weisberg [1,2].

Key to Symbols:

°	Triaxial coordinates
αH^+	Hydrogen ion activity
A	Acid, expressed as ± Pco_2 (Kintner)
Alk. Res.	Alkaline reserve (McClendon)
B	Base, expressed as ± base (⇔BE) (Kintner)
BB	Buffer base
BE	Base excess
BE_{WB}	Base excess, whole body (Russell *et al.*)
BE/D_{ECF}	Base excess/deficit of extracellular fluid (Siggaard-Andersen)
BE/D_{Hb5}	Base excess/deficit for extracellular fluid with hemoglobin at 5 g/dl (Rooth)
C_{H^7}	Hydrogen ion concentration (⇔pH)
CSF	Cerebrospinal fluid (McClendon)
CT	Total carbon dioxide (as mmol/l or vol %, etc.)
CY	CO_2 capacity (Goldberger)
ΔA-B	Δ Acid-base (Astrup)

Table 2. Comparison of 105 acid-base diagrams (continued).

ECF	Extracellular fluid
$[H^+]$	Hydrogen ion concentration
H^+_{40}	Hydrogen at 40 mmHg (Frumin)
$[HCO_3^-]$	Bicarbonate ion concentration
$[HCO_3^-]_{40}$	Bicarbonate at 40 mmHg (Siggaard-Andersen); as SB
$[H_2CO_3]$	Carbonic acid (and dissolved CO_2) concentration
LA	Lactic acid
N	Normality of H^+ (McClendon)
"N"	Normality of titration of alkaline reserve (McClendon)
$[O_2]$	Concentration of (dissolved) O_2
O_2 CT	Total oxygen (vol %)
Pco_2	Partial pressure of carbon dioxide
"Pco_2"	Assumed from % CO_2 in a alveolar air (McClendon)
pH_{40}	pH at 40 mmHg (Kappagoda *et al.*)
PR	Serum protein (Stadie *et al.*)
R	Ventilation ratio (Kim *et al.*)
S	Solubility coefficient for CO_2 in plasma at 38 °C
SB	Standard bicarbonate at Pco_2 40 mmHg, 38 °C, Hb oxygenated
$SB_{7.40}$	Standard bicarbonate at pH 7.40 (Van Slyke)
t°	Temperature (Stadie *et al.*) (Lenfant)
T_{40}	Bicarbonate at 40 mmHg *in vivo* (Collier *et al.*)
TB	Total buffer (Coats)
VR	Ventilation ratio
WB	Whole body

		Reference		Values Plotted On					
Year	Author(s)	1°	2°	Ordinate	Log?	Abscissa	Log?	Others	Log?
1914	Christiansen *et al.*	3	4,5	CT		Pco_2		-	
1916	Lewis *et al.*	6	7	pH		CT		-	
1916	McClendon	8		Pco_2		pH CT		-	
1917	McClendon *et al.*	9		Pco_2	Yes	pH $[H+]$	Yes	-	
1917	McClendon	10		pH $[H^+]$	Yes	Pco_2	Yes	$[HCO_3^-]$	Yes
1917	McClendon	10		CT		pH		Alk. Res.	Yes
1917	McClendon *et al.*	11		Pco_2	Yes	$[H^+]$ pH	Yes	Alk. Res.	Yes
1917	Parsons	12	13	pH		Pco_2			

Table 2. Comparison of 105 acid-base diagrams (continued).

Year	Author(s)	Reference 1°	Reference 2°	Values Plotted On: Ordinate	Log?	Abscissa	Log?	Others	Log?
1917	Parsons	12	13-15	$[H^+]$		Pco_2		-	
1918	McClendon	16		"Pco_2"	Yes	$[H^+]$ pH	Yes	CSF "N"	Yes
1918	Straub & Meier	17		CT		Pco_2		pH $[H_2CO_3]$	Yes
1919	Haggard & Y. Henderson	18		CT		Pco_2		C_{H^7} $[H_2CO_3]$	Yes
1920	Henderson	19		$[HCO_3^-]$		$[H^+]$		-	
1921	Henderson	20		$[HCO_3^-]$		pH		$[H_2CO_3]$ $[Cl^-]$ Po_2 HbO_2	
1921	Van Slyke	21	22-24	CT		Pco_2		pH $[H_2CO_3]$	Yes
1921	Van Slyke	21		$[HCO_3^-]$		pH		-	
1922	Van Slyke *et al.*	25		CT $[HCO_3^-]$		pH		-	
1922	Doisy *et al.*	26		pH		$[HCO_3^-]$		-	
1923	Peters *et al.*	27	28	$[HCO_3^-]$	Yes	$[H_2CO_3]$	Yes	-	
1923	Peters	29		CT	Yes	Pco_2	Yes	pH	Yes
1923	Peters	29	24	CT	Yes	Pco_2 $[H_2CO_3]$	Yes Yes	pH	
1923 1924-25	Cullen & Jonas Hastings *et al.*	30 31	32,33,193	CT		pH		Pco_2	Yes
1924	Bock *et al.*	28	23	$[HCO_3^-]$		pH		-	
1924	Bock *et al.*	28		CT		pH		-	
1925	Murray & Hastings	34		$[HCO_3^-]$	Yes	pH		$[H_2PO_4^-]$ $[HPO_4^{2-}]$ $[PO_4^{3-}]$ $[Ca^{2+}]$ $[CO_3^{2-}]$	Yes Yes Yes Yes Yes
1925	Austin & Cullen	35		$[HCO_3^-]$		Pco_2		$[H_2CO_3]$	
1925	Austin & Cullen	35	36	CT		pH		Pco_2	Yes

Table 2. Comparison of 105 acid-base diagrams (continued).

Year	Author(s)	Reference 1°	Reference 2°	Values Plotted On: Ordinate	Log?	Abscissa	Log?	Others	Log?
1925	Stadie *et al.*	37		$[HCO_3^-]$ PR		pH		-	
1925	Stadie *et al.*	37		t°		pH		$[HCO_3^-]$ $[H_2CO_3]$ Pco_2	Yes Yes Yes
1927	Eisenman	38		CT	Yes	Pco_2	Yes	-	
1927	Dill *et al.*	39		CT	Yes	Pco_2	Yes	-	
1928	Henderson	36		CT O_2 CT $[H_2CO_3]$ $[O_2]$		Pco_2 Po_2		-	
1928	Henderson	36		Pco_2		pH		CT	Yes
1931	Peters & Van Slyke	23	40	CT	Yes	Pco_2	Yes	pH	
1931	Hastings & Steinhaus°	41	42-46	Pco_2	Yes	pH		$[HCO_3^-]$	Yes
1932	Douglas & Havard	47		pH CT		Pco_2		-	
1935	McClendon°	48		pH		Pco_2	Yes	$[HCO_3^-]$	Yes
1947	Davenport	49	45,50-54	$[HCO_3^-]$		pH		Pco_2	Yes
1948	Clark	55	56-61,165	$[HCO_3^-]$		Pco_2		pH	Yes
1951	Singer	62		BB		Pco_2		CT pH	Yes Yes
1954	Astrup	7	63,69,77,164	Pco_2	Yes	pH		-	
1955	Cranston *et al.*	64		$[H^+]$		$[H_2CO_3]$		-	
1956	Roberts *et al.*	65		Pco_2 pH		CT		-	
1956	Poppel *et al.*	66	67,68	Pco_2		CT		-	
1956	Astrup	69	70	Pco_2	Yes	pH		SB	Yes
1958	Winters *et al.*	71	72	CT	Yes	pH		Pco_2	Yes
1958	Jørgensen	73		SB	Yes	Pco_2	Yes	pH	
1959	Goldberger	74		CT CY		pH		Pco_2	
1959	Peirce	75		Pco_2		pH		BB	Yes

Table 2. Comparison of 105 acid-base diagrams (continued).

Year	Author(s)	Reference 1°	Reference 2°	Values Plotted On: Ordinate	Log?	Abscissa	Log?	Others	Log?
1959	Astrup	76		Pco_2	Yes	pH		BB SB ΔA-B	Yes Yes Yes
1960 1960	Astrup *et al.* Siggaard-Andersen	77 78	46,52, 78-89	Pco_2	Yes	pH		BB SB BE	Yes Yes Yes
1960	de la Huerga	90, 91		CT		pH	Yes	Pco_2	
1961	Weisberg	92		CT		$[H^+]$ pH	 Yes	Pco_2	Yes
1961	Lenfant	93		$[H_2CO_3]$	Yes	pH		CT	Yes
1961	Lenfant	93		$[H_2CO_3]$	Yes	Pco_2		t°	
1961	Weisberg	92	1	CT		pH $\frac{[HCO_3^-]}{[H_2CO_3]}$	 Yes	Pco_2	Yes
1962	Campbell	94	95,96	Pco_2 $[H_2CO_3]$		$[HCO_3^-]$		$[H^+]$ pH	Yes Yes
1962	Campbell	94	97	Pco_2 $[H_2CO_3]$	Yes Yes	$[HCO_3^-]$	Yes	αH^+ [H+] pH	Yes Yes
1962	Nunn	98		$[HCO_3^-]$		Pco_2		pH	Yes
1963	Siggaard-Andersen	81	99	Pco_2	Yes	pH		$[HCO_3^-]$	Yes
1963	Weisberg (revised)	100	101-103	CT		pH $[H^+]$ $\frac{[HCO_3^-]}{[H_2CO_3]}$	 Yes Yes	Pco_2	Yes
1963	Robin	104		pH		Pco_2		$[HCO_3^-]$	Yes
1963	Fink & Nahas	105		$[H^+]$ pH	 Yes	Pco_2		$[HCO_3^-]$ VR	Yes
1964	Whitehead	106, 107		Pco_2	Yes	$[H^+]$	Yes	-	
1964	Coats	108		$[HCO_3^-]$		$[H_2CO_3]$		pH TB	Yes
1964	Darrow	109		$[HCO_3^-]$		Pco_2 $[H_2CO_3]$		pH $\frac{[HCO_3^-]}{[H_2CO_3]}$	Yes Yes
1965	Woodbury	110	15,52, 111-114	$[HCO_3^-]$		pH $[H^+]$	 Yes	Pco_2	Yes

Table 2. Comparison of 105 acid-base diagrams (continued).

Year	Author(s)	Reference 1°	Reference 2°	Values Plotted On: Ordinate	Log?	Abscissa	Log?	Others	Log?
1965	Schwartz (*et al.*)	115, 116	13,117, 118	$[HCO_3^-]$		Pco_2		-	
1965	Schwartz (*et al.*)	115, 116	117-119	$[H^+]$ pH	Yes	Pco_2		-	
1965	Mithoefer *et al.*	120	121	$[HCO_3^-]$	Yes	Pco_2	Yes	$[H^+]$ pH	Yes
1965	Owen *et al.*	122		Pco_2		$[H^+]$ pH	 Yes	$[HCO_3^-]$	
1965	Elkinton	123	124	$[HCO_3^-]$	Yes	Pco_2	Yes	pH	
1965	Siesjö & Ponten	125		Pco_2	Yes	$\frac{[HCO_3^-]}{S \cdot Pco_2}$	Yes	SB	Yes
1966	Instrumentation Lab°	126		Pco_2	Yes	pH		CT	Yes
1966	Lennon & Lemann	14		$[H^+]$		$[HCO_3^-]$		-	
1966	Lennon & Lemann	14	127,128, 140	Pco_2		$[HCO_3^-]$		-	
1966	Kim *et al.*	129		CT		Pco_2		pH R O_2 CT	Yes
1966	Stinebaugh & Austin	130	131,132	Pco_2		CT		pH	Yes
1966	Young	133		$[HCO_3^-]$	Yes	pH $[H^+]$	 Yes	Pco_2	Yes
1967	Cohen	134	135-138	$[H^+]$ pH	 Yes	Pco_2		$[HCO_3^-]$	Yes
1967	Albert *et al.*	127		Pco_2		BE		-	
1967	Albert *et al.*	127	139	Pco_2		$[H^+]$		-	
1967	Albert *et al.*	127		Pco_2		pH		-	
1967	Siggaard-Andersen	85	89,141	Pco_2	Yes	pH $[H^+]$	 Yes	$BE_{(ECF)}$ $[HCO_3^-]_{40}$	Yes Yes
1967	Kintner	142		CT		pH $[H^+]$	 Yes	Pco_2 A B	Yes Yes Yes
1967	Winters	57	59	Pco_2		$[HCO_3^-]$		pH	Yes
1967	Winters	57	59,143	Pco_2		BE		pH	Yes
1967	Winters	57	59	BE		Pco_2		pH	Yes

Table 2. Comparison of 105 acid-base diagrams (continued).

Year	Author(s)	Reference 1°	Reference 2°	Values Plotted On: Ordinate	Log?	Abscissa	Log?	Others	Log?
1967	Srouji	144		BE		Pco_2		-	
1968	Engel *et al.*	13		BE		Pco_2		-	
1968	Goldring *et al.*	145	146	pH		$[HCO_3^-]$		Pco_2	Yes
1969	Li & Holder	147		pH		SB		Pco_2	Yes
1969	Nunn	112		Pco_2	Yes	pH $[H^+]$	 Yes	-	
1969	Weisberg	148	149-153	CT		pH $[H^+]$ $\frac{[HCO_3^-]}{[H_2CO_3]}$	 Yes Yes	Pco_2 $[H_2CO_3]$	Yes Yes
1970	Gilbert & Auchincloss	154		Pco_2		$[H^+]$		-	
1970	Heisler & Schorer	155	89	$[HCO_3^-]$		pH		Pco_2 BB BE	Yes Yes Yes
1970	Kappagoda *et al.*	156		Pco_2	Yes	pH		pH_{40}	
1970	Rooth	157		Pco_2		BE_{Hb_5} BD_{Hb_5}		pH	Yes
1970	Rooth	157		BE_{Hb_5} BD_{Hb_5}		Pco_2		pH	Yes
1970	Blair	158		Pco_2 BE LA		pH		-	
1971	Stephens	159		CT		pH	Yes	Pco_2	Yes
1971	Slonim & Hamilton	160		$[HCO_3^-]$		Pco_2 $[H_2CO_3]$ VR	 Yes	pH	
1972	Kintner	161		$[HCO_3^-]$		pH		Pco_2 BB	Yes
1972	Russel *et al.*	162		Pco_2	Yes	pH		BE_{WB}	Yes
1973	Visser	163		Pco_2	Yes	pH		$SB_{7.40}$	Yes

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THE OVERALL FIRST IONIZATION EQUATION OF CARBONIC ACID AS RELATED TO CO_2 IN GAS PHASE: A NEW pK

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For the evaluation of acid-base disturbances in biological fluids, the carbonic acid/bicarbonate buffer system is in common use. In all studies this system is described by the 1st ionization equation of carbonic acid as related to the physically dissolved carbon dioxide in the solution: the well-known Henderson-Hasselbalch equation. As a result of various applications, originally for calculation of the pH of plasma, later on the pCO_2 and nowadays the actual bicarbonate concentration, standard bicarbonate concentration or total-CO_2 concentration, this equation is written in different forms. However, little emphasis has been placed on an exact formulation which accounts for the non-ideality of the system. By virtue of the thermodynamic theory of equilibrium, we have shown that the 1st ionization equilibrium of carbonic acid, which relates the bicarbonate concentration and the partial pressure of CO_2 to the pH of the solution, is preferable to the conventional Henderson-Hasselbalch equation [1][1].

1. Theory

If there is equilibrium between CO_2 in the gas phase and that in the liquid phase, we can consider the overall equilibrium:

$$CO_2\ (gas) + H_2O \rightleftarrows HCO_3^- + H^+ \qquad (1)$$

of which the equilibrium constant K_{1g} is defined as:

$$K_{1g} = \frac{aH^+ \cdot aHCO_3^-}{aH_2O \cdot fCO_2} = \frac{yHCO_3^-}{aH_2O \cdot gCO_2} \times \frac{aH^+ \cdot cHCO_3^-}{pCO_2} \quad . \qquad (2)$$

Suffix g in K_{1g} refers to CO_2 in gas, a, f, y, g, c and p symbolize respectively, activity, fugacity, activity coefficient, fugacity coefficient, concentration, and partial pressure.

For practical purpose we define a practical coefficient K'_{1g}:

$$K'_{1g} = \frac{aH_2O \cdot gCO_2}{yHCO_3^-} \times K_{1g} = \frac{aH^+ \cdot cHCO_3^-}{pCO_2} \qquad (3)$$

[1]Figures in brackets indicate the literature references at the end of this paper.

Usually one considers the 1st ionization equilibrium as related to the sum of freely dissolved CO_2 and the hydrated CO_2. The starting-point in that case is the overall 1st ionization equilibrium, related to the dissolved carbon dioxide:

$$CO_2(\text{liquid}) + H_2O \rightleftarrows H^+ + HCO_3^- \tag{4}$$

of which, the equilibrium constant is defined as follows:

$$K_{1_1} = \frac{aH^+ \cdot aHCO_3^-}{aH_2O \cdot aCO_2} = \frac{yHCO_3^-}{aH_2O \cdot yCO_2} \times \frac{aH^+ \cdot cHCO_3^-}{cCO_2} \quad . \tag{5}$$

The suffix 1 in K_{1_1} refers to CO_2 in the liquid phase. Now the CO_2 concentration is equal to the solubility S times pCO_2 minus the concentration of H_2CO_3:

$$cCO_2 = S \cdot pCO_2 - cH_2CO_3 \tag{6}$$

which equation by means of the hydration constant K_h can be written as:

$$cCO_2 = S \cdot pCO_2/(1 + \frac{aH_2O \cdot yCO_2 \cdot K_h}{yH_2CO_3}) \tag{7}$$

or in a simplified form:

$$cCO_2 = S \cdot pCO_2/(1 + \Delta) \quad . \tag{8}$$

If we replace cCO_2 by $S \cdot pCO_2/(1 + \Delta)$ in equation (5) then K_{1_1} is changed into the conventional form:

$$K_{1_1} = \frac{yHCO_3^- \cdot (1 + \Delta)}{aH_2O \cdot yCO_2} \times \frac{aH^+ \cdot cHCO_3^-}{S \cdot pCO_2} \quad . \tag{9}$$

For practice we again define a coefficient easy in operation:

$$K'_{1_1} = \frac{aH_2O \cdot yCO_2}{yHCO_3^- \cdot (1 + \Delta)} \times K_{1_1} = \frac{aH^+ \cdot cHCO_3^-}{S \cdot pCO_2} \quad . \tag{10}$$

To be able to choose between the two practical equations we first write them in the usual form. We take the negative logarithm of these equations and apply the notation -log = p. This gives us for equation (10):

$$paH^+ = pK_{1_1} - \log \frac{aH_2O \cdot yCO_2}{yHCO_3^- \cdot (1 + \Delta)} + \log \frac{cHCO_3^-}{S \cdot pCO_2} = pK'_{1_1} + \log \frac{cHCO_3^-}{S \cdot pCO_2} \tag{11}$$

which equation represents the exactly defined expression of what is now known as the Henderson-Hasselbalch equation and for equation (3):

$$p_aH^+ = pK_{1g} - \log \frac{a_{H_2O} \cdot g_{CO_2}}{y_{HCO_3^-}} + \log \frac{a_{HCO_3^-}}{p_{CO_2}} = pK'_{1g} + \log \frac{a_{HCO_3^-}}{p_{CO_2}} \qquad (12)$$

which equation we propose to call the modified Henderson-Hasselbalch equation. Since this equation contains one variable less, namely the solubility coefficient S, this modification of the Henderson-Hasselbalch equation is the most suitable equation for description of the carbonic acid/bicarbonate buffer system. Assuming g_{CO_2} equals 1 (see ref [1]) and Δ equals zero, it will be clear that the variability of pK'_{1g} is determined by the ratio $a_{H_2O}/y_{HCO_3^-}$ and the variability of pK'_{1_1} overmore by y_{CO_2}, which coefficient strongly depends on lipid content of the solution.

2. Experimental Section

In order to be able to apply the 1st ionization equilibrium of carbonic acid as related to CO_2 in gas, we established the values of the ionization constant (pK_{1g}) and practical coefficient ($p\tilde{K}'_{1g}$) over a large range of application [2].

A. pK_{1g}

The true ionization constant pK_{1g} has been determined in water using ($NaHCO_3$ + NaCl) solutions of ionic strength $0.01 < I < 0.500$ in equilibrium with (CO_2 + H_2) gas mixtures applying electromotive force measurements with a hydrogen electrode cell without a salt bridge from which the exact pK_{1g} value results. We found:

$$pK_{1g}\ (25°C) = 7.720 \qquad pK_{1g}\ (38°C) = 7.810 \ .$$

pK_{1g}, a thermodynamic constant, is independent of solution composition and varies with temperature only. For the temperature range 25-40 °C, a useful equation could be derived subtracting the equation

$$pK_{1_1} = \frac{3374.162}{T} + 0.03272T - 14.707 \qquad (13)$$

from Shedlovsky and McInnes [3] and

$$\log S_o = \frac{2351.76}{T} + 0.0148483T - 13.670 \qquad (14)$$

from data of Bartels [4] and Austin [5] for pure water resulting in the relationship:

$$pK_{1g} = pK_{1_1} - \log S_o = \frac{1022.404}{T} + 0.01787T - 1.037 \qquad (15)$$

where T is the absolute temperature. Our measured data agree within 0.005.

B. $p\tilde{K}'_{1g}$

The practical coefficient

$$p\tilde{K}'_{1g} = p\tilde{K}_{1g} - \log \frac{a H_2O}{y HCO_3^-} = pH - \log c HCO_3^- + \log p CO_2 \qquad (16)$$

was determined in ($NaHCO_3$ + NaCl) solutions, cerebrospinal fluid (CSF), plasma and serum, measuring pH, $cHCO_3^-$ and pCO_2. We use the symbol ~ to indicate that pH in consequence of the residual liquid junction potential in the measurement is not exactly identical to paH^+. $p\tilde{K}_{1g}$ and $p\tilde{K}'_{1g}$ are related to pK_{1g} and pK'_{1g} according to the following equation

$$p\tilde{K}_{1g} - pK_{1g} = p\tilde{K}'_{1g} - pK'_{1g} = pH - paH^+ . \qquad (17)$$

3. Bicarbonate Solutions

Using a hydrogen electrode cell including a salt bridge, we measured the practical coefficient for various ionic strengths and found by extrapolating to infinite dilution an approximate value of the ionization constant $p\tilde{K}_{1g}$, which differed *circa* 0.005 from pK_{1g} indicating that the residual liquid junction is very small. From these measurements, we also derived an empirical equation for $yHCO_3^-$, which in the form as recalculated by Siggaard-Andersen [6] reads:

$$\log y HCO_3^- = -(0.50 + 0.001\Delta T)\sqrt{I} + 0.13I \qquad (18)$$

where $\Delta T = T - 288.15$ K.

As a first approximation, log aH_2O decreases linearly with I according to the equation [2]:

$$\log a H_2O = -0.015I \qquad (19)$$

Combining equations (15), (18), (19) and adding 0.005 for the residual liquid junction potential give a valid approximation of $p\tilde{K}'_{1g}$ as a function of ionic strength at temperatures 15-40 °C.

$$p\tilde{K}'_{1g} = \frac{1022.404}{T} + 0.01787T - 1.042 - (0.50 + 0.001\Delta T)\sqrt{I} + 0.145I . \qquad (20)$$

Our measured results deviate less than 0.005. For the activity coefficient yH^+, we recalculated our data and found the relationship:

$$\log yH^+ = -(0.50 + 0.001\Delta T)\sqrt{I} + 0.81 . \qquad (21)$$

Apart from the problem of the residual liquid junction potential, the usual glass electrode deviates from the fundamental hydrogen electrode in the pH determination [7].

Further, the bicarbonate determinations in body fluids are subject to error of analysis, because carbonate and carbamate are enclosed in the present titrimetric and gasometric analysis [8]. To be able to understand quantitatively the influence of the disturbing "bicarbonate" fractions and the error of the glass electrode, we have determined the practical coefficients $p\tilde{K}'_{lg}$ of a bicarbonate solution isotonic with CSF and plasma, composition 0.025 mol/l $NaHCO_3$ + 0.135 mol/l NaCl over the range 6-8 pH and performed simultaneously the required pH measurements with the hydrogen electrode cell as well as with the glass electrode cell at 25 and 38 °C (fig. 1).

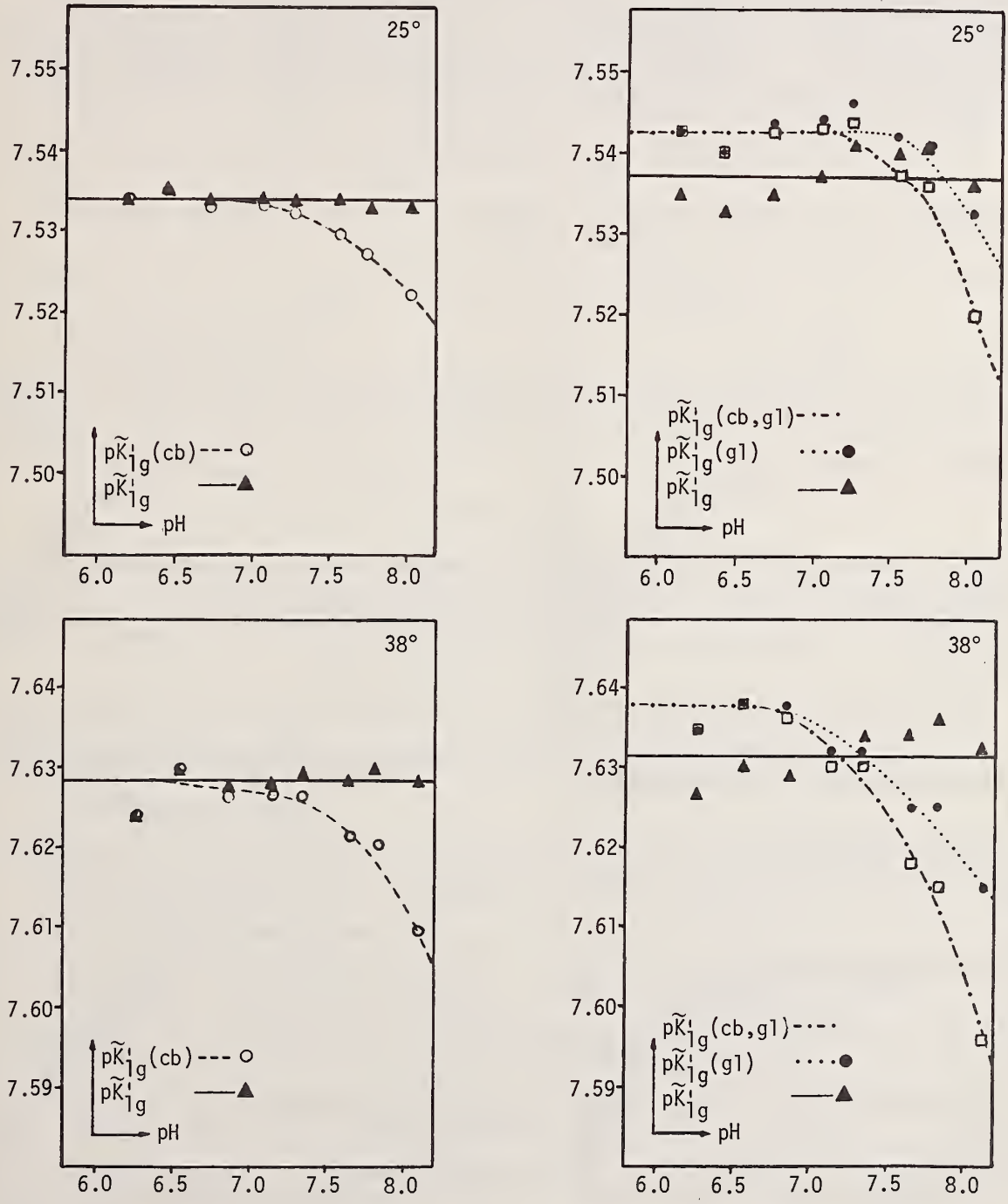

Figure 1. The uncorrected and corrected practical coefficient $p\tilde{K}'_{lg}$ of ($NaHCO_3$ + NaCl) solution (I = 0.16), determined with the hydrogen electrode cell and with the glass electrode cell as a function of pH (see text).

In the two graphs on the left-hand side, the overall 1st ionization coefficient, determined with the hydrogen electrode cell, is plotted as a function of the pH. The broken line indicates the uncorrected coefficient $p\tilde{K}'_{1g}$ (cb), and the drawn line the coefficient $p\tilde{K}'_{1g}$ corrected for the carbonate error. In the graphs on the right-hand side, the coefficient, determined with a cell with the glass electrode is plotted as a function of the pH. The broken line gives the uncorrected coefficient $p\tilde{K}'_{1g}$ (cb, gl), the dotted line the coefficient $p\tilde{K}'_{1g}$ (gl) corrected for the carbonate error and the straight line the coefficient $p\tilde{K}'_{1g}$ corrected for the carbonate error and the glass electrode. These experiments affirm that the $p\tilde{K}'_{1g}$ does not vary with the pH, as is established by definition. On the other hand, Siggaard-Andersen [9] explains the variation of the $p\tilde{K}'_{1g}$ with the pH he found, by the presence of non-dissociated $NaCO_3^-$ besides CO_3^{2-} ions. His experiments have been carried out with a glass electrode of the type used by us. By applying the right correction, however, we have come to the conclusion that the variation of $p\tilde{K}'_{1g}$ (gl) with the pH should be explained from the property of the glass electrode itself.

4. CSF, Plasma and Serum

Altogether, of 15 samples of CSF and 12 samples of plasma and sera the coefficient $p\tilde{K}'_{1g}$ (cb, gl) was determined at 25 °C and 38 °C.

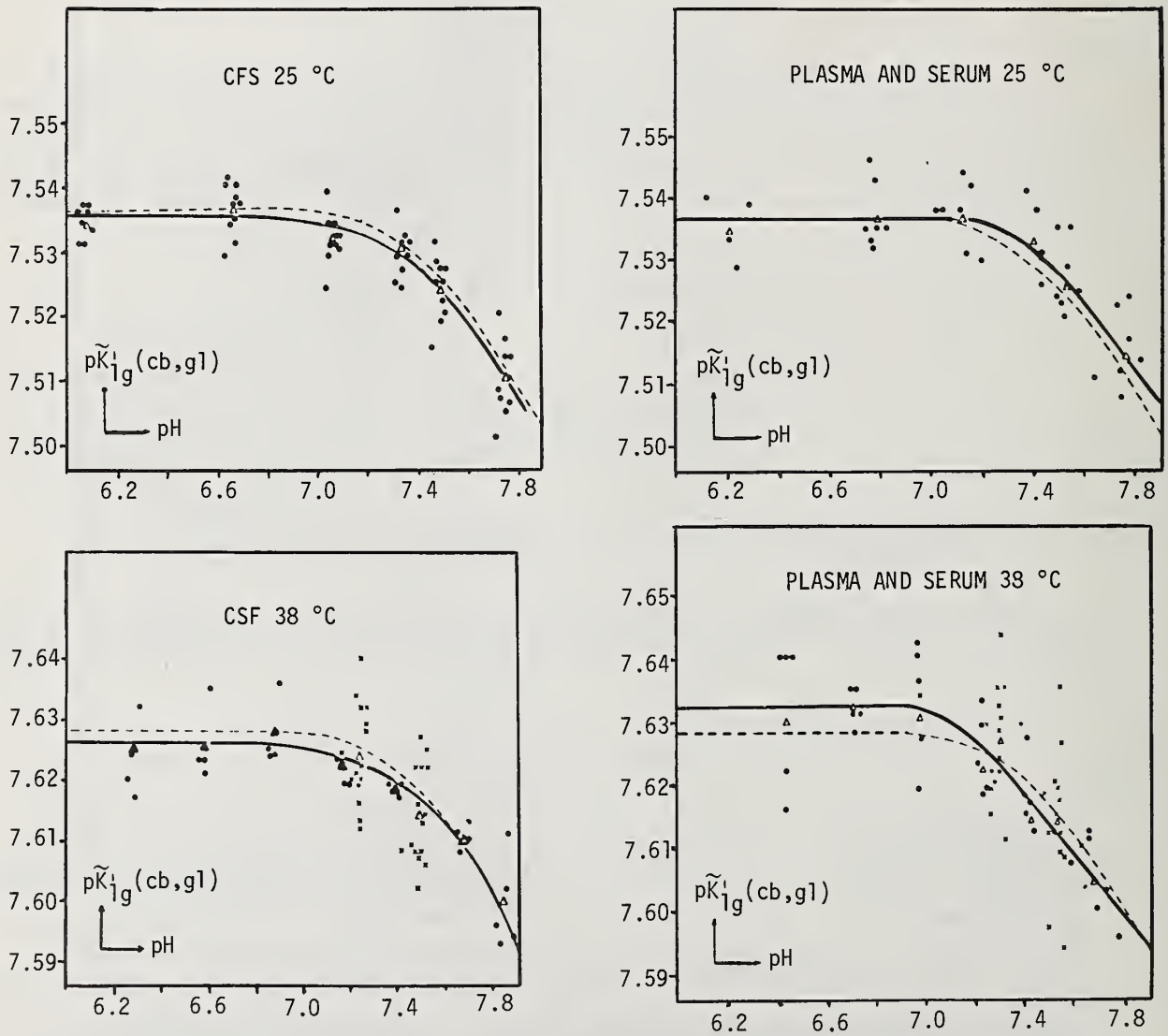

Figure 2. The practical coefficient $p\tilde{K}'_{1g}$ (cb, gl) of CSF and plasma (serum) as a function of pH (see text).

group of $p\tilde{K}'_{1g}$ (cb, gl) we have taken the mean value, indicated by Δ. Through it, we have drawn a line at sight. At pH smaller than 7, $p\tilde{K}'_{1g}$ (cb, gl) is constant, and at pH higher than 7, $p\tilde{K}'_{1g}$ (cb, gl) decreases. This decrease is the same as that of the discussed bicarbonate solution. From this, we may conclude that the dependence of this practical coefficient on the pH in the physiologic pH range must be due to the influence of the 2nd dissociation of carbonic acid on the bicarbonate determination and the non-ideal cell with the glass electrode for the pH determination.

The $p\tilde{K}'_{1g}$ (cb, gl) values of CSF (7.527 at 25 °C and 7.617 at 38 °C) and plasma (7.529 at 25 °C and 7.624 at 38 °C) do not appear to differ significantly, indicating that the ionic strength of both fluids is equal. So it is also misleading to use the old values $p\tilde{K}'_{11}$ (cb, gl) = 6.13 for CSF and $p\tilde{K}'_{11}$ (cb, gl) = 6.10 for plasma (at 38 °C), suggesting that the ionic strength of plasma is higher than that of spinal fluid. For simplicity we propose to take a mean quantitity for CSF and plasma, to be designated by the asterisk, pK^*_{1g}. In the graphs pK^*_{1g} is drawn as a function of the pH (dotted line). Finally, we deduced from eq. (15) and the formula of mentioned dotted line [2] an approximation for pK^*_{1g} valid valid in CSF and plasma over the pH range of 6.0-7.8 pH at temperature 15-40 °C.

$$pK^*_{1g} = \frac{1022.401}{T} + 0.01787T \qquad (22)$$

$$- 1.21813 - 0.0012(pH-7.0) - 0.0406(pH-7.0)^2$$

The precision of the pK^*_{1g} value obtained for CSF or plasma of normal subjects, is better than 0.01 pK^*_{1g}. Extreme pathological variation in the ionic strength of the plasma, for example I = 0.160 ± 0.030, causes a variation in pK^*_{1g} of about ± 0.015 as verified with eq. (20).

5. Conclusion

On theoretical and practical grounds, we recommend the use of the practical equation of the overall 1st ionization equilibrium of carbonic acid as related to the gas phase for calculations of the bicarbonate concentration (or total CO_2 content) of CSF or plasma. At pH = 7.40 and t = 37 °C the pK^*_{1g} value is equal to 7.615 for CSF, plasma and serum.

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TOWARDS A PHYSIOLOGIC NOMENCLATURE FOR *IN VIVO* DISTURBANCES OF ACID-BASE BALANCE

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In considering disturbances of acid-base equilibrium from a clinical vantage point, it would seem axiomatic that a fundamentally physiological frame of reference must dominate any conceptual scheme offered as an aid to diagnosis and therapy. It is abundantly clear that both normal physiologic regulation as well as patho-physiologic disturbances of acid-base equilibrium in the living organism involve a large array of complexly interacting, dynamic processes. Although the simplest of these processes, that of buffering, can for some purposes be conceived of in strictly chemical terms, a thorough analysis of even this process must incorporate many features which are strictly within the domain of physiology and which are not adequately encompassed by purely chemical notions. I refer here to such features as compartmentalization, recruitment, endocrine influences, "slow equilibration," membrane transport, and the like. Add to this the physiologic "regulatory" functions of the respiratory and renal systems, each of which is impinged upon by a veritable welter of influences, and one can scarcely deny that true comprehension of this system poses an enormous challenge.

As has been amply discussed, this challenge cannot be circumvented by systems of acid-base analysis based exclusively on the *in vitro* behavior of blood [1][1]. There would appear, in fact, to be no alternative to using the empiric behavior of the intact living organism itself, as the foundation for any systematic and satisfactory analysis of acid-base metabolism.

Beginning with this assumption, with which I would hope we could all agree, our task would appear to be to develop a nomenclature commensurate with the physiologic complexities that are known to exist. As a point of departure for developing such a nomenclature, there are compelling reasons for utilizing the time-honored, carbonic acid/bicarbonate buffer pair. As we all know, this buffer pair occupies a unique position in the living organism since it provides the biologic linchpin connecting the chemical process of buffering, on the one hand, to the physiologic process of acidity regulation, on the other. This unique property stems, of course, from the fact that carbonic acid and bicarbonate ion concentrations are regulated by semi-independent physiologic control systems. Moreover, extrapolating directly from the isohydric principle, one can state that alterations in body fluid acidity can only occur as a consequence of alterations either in carbonic acid or in bicarbonate concentrations (or both). Disturbances of acidity can be classified with little ambiguity, therefore, into "respiratory" and "metabolic"[2] subgroups.

Considerably more ambiguity is encountered, however, in attempting to develop terms which connote the directional changes in acidity that are produced by these two classes of disturbances. In the first place, there is the difficulty encountered with respect to the secondary changes in bicarbonate and carbonic acid concentration which are evoked by primary disturbances in the countervening variable. For example, should the secondary hyperventilization induced by an initial reduction in bicarbonate concentration be given a separate term such as "respiratory alkalosis" when it is part and parcel of the organisms

[1]Figures in brackets indicate the literature references at the end of this paper.

[2]Although the term "metabolic" is somewhat narrow in scope in that it fails to communicate adequately the frequently overriding influence of renal mechanisms on bicarbonate concentration, it is far too well engrained to be easily supplanted.

inherent response to this challenge and when, in fact, the patient remains acidemic. Even more troublesome is the difficulty encountered in certain mixed acid-base disturbances in which the interaction of more than one primary disturbance, and their respective physiologic responses, produces truly paradoxical deviations in acidity. For example, we have recently described an experimental situation in which chronic hyperventilation causes a significant elevation in hydrogen ion concentration [2].

Based on the considerations that I have all too briefly summarized above, I would offer the following set of recommendations for a physiologic nomenclature adequate to deal with *in vivo* disturbances of acid-base balance.

A. The acid-base status of an individual at a given moment in time should be characterized *only* by:

1) a direct measure of the "respiratory" component, *i.e.*, carbonic acid concentration (H_2CO_3) or carbon dioxide tension (PCO_2) and

2) a direct measurement of the "metabolic" component, *i.e.*, bicarbonate concentration (HCO_3^-).

B. The level of blood acidity, which is determined by the levels of the co-existing respiratory and metabolic components, should be quantitated in terms of the actual hydrogen ion activity, symbolized by pH or $[H^+]$ or a_{H^+}.

C. Deviations from normal acidity, irrespective of cause, should be termed "acidemia" and "alkalemia," respectively.

D. Deviations from the normal level of the respiratory component, irrespective of cause, should be termed "hypocapnia" and "hypercapnia," respectively.

E. Deviations from the normal level of the metabolic components should be termed "reduced bicarbonate concentration" (? hypobicarbia) and "elevated bicarbonate concentration" (? hyperbicarbia), respectively.

F. Disturbance of acidity *initiated* by changes in the respiratory component should be termed "respiratory alkalosis" and "respiratory acidosis," respectively. Changes in the respiratory component occurring in response to primary changes in the metabolic component should be termed "secondary hyperventilation" and "secondary hypoventilation," respectively, and should *not* be termed respiratory alkalosis and respiratory acidosis.

G. Disturbances of acidity *initiated* by changes in the metabolic component should be termed "metabolic acidosis" and "metabolic alkalosis," respectively. Changes in the metabolic component occurring in response to primary changes in the respiratory component should be termed "secondary elevations in bicarbonate concentration" and "secondary reductions in bicarbonate concentration," respectively, and should *not* be termed metabolic alkalosis and metabolic acidosis.

H. For the respiratory disturbances, the adjectives "hyper-acute," "acute," "transient," and "chronic" should be used to designate the duration of the acid-base disturbance with respect to the secondary physiologic adjustments in bicarbonate concentration.

1) "Hyper-acute"--the virtually instantaneous, and largely theoretical, interval prior to the titration of tissue buffers.

2) "Acute"--the interval following the immediate titration of tissue buffers and prior to a measurable renal contribution.

3) "Transient"--the period during which the renal contribution is accumulating.

4) "Chronic"--the indefinite period following completion of the renal contribution to the new-steady state.

I. The adjective "uncomplicated" should be used to designate an abnormal state of acid-base equilibrium produced by a single primary disturbance, including the influence of

the secondary physiologic response appropriate to the specific degree and duration of the primary change.

J. The adjective "mixed" should be used to designate a state of acid-base equilibrium produced by the simultaneous presence of more than one, independent disturbance.

K. The terms "correction" and "repair" should be used to designate the restoration to normal levels of the component responsible for initiating an acid-base disturbance.

It should be noted that the terms "compensated" and "uncompensated" have been avoided in this scheme since these terms are commonly misconstrued to mean success and failure, respectively, in returning the level of acidity to normal; it is now abundantly clear that unimpeded physiologic responses evoked by initiating disturbances of acid-base equilibrium should not be expected to restore hydrogen ion concentration to control levels.

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MINIMAL ACCEPTANCE CRITERIA FOR ACID-BASE NOMOGRAMS

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Acid-base nomograms can be classified into two major subgroups: (a) those providing a graphic representation of the chemical equilibrium of the carbonic acid-bicarbonate and/or other buffer systems, and (b) those concerned with delimiting the range of physiologic responses to be anticipated, on empiric grounds, in response to primary disturbances of acid-base equilibrium of a specified magnitude.

The first class of nomogram has proven useful as a convenient means for specifying the value of one (unmeasured) variable when other components of the equilibrium reaction have been independently analyzed. These nomograms are also helpful in the teaching of acid-base physiology since they can often be used as a backdrop for the visual representation of the various pathways of disturbances. Such nomograms have also, on occasion, been modified and extended to permit evaluation of certain "derived" parameters which have been offered as aids to the analysis of clinical disturbances of acid-base equilibrium. To the extent that such nomograms reflect accurately the actual chemical equilibrium upon which they are based, one can certainly have no quarrel with their use as handy, calculational aids. It is only when such nomograms make available certain derivative acid-base parameters that are subsequently misused or misinterpreted by the unsophisticated that problems arise. Clearly, this is not an indictment of such nomograms themselves, but does raise certain risk-benefit considerations that might provide a useful focus for our discussion. To put my own bias squarely on the table, let me say that I can find no compelling reason for developing nomograms or algorithms that permit the calculation of so-called derived parameters since an adequate and straightforward analysis of acid-base disturbances can be based directly on the actual concentrations of bicarbonate and carbonic acid. To state the issue in a slightly different way, the information content required to analyze a clinical acid-base disturbance is no less when using derived parameters than when using the actual values for bicarbonate and carbonic acid (PCO_2) themselves.

The second class of nomograms, that is concerned with delimiting the empiric physiologic ranges for the anticipated responses to a given acid-base disturbance, has gained wide popularity in recent years. This type of nomogram has, of course, been made possible owing to the availability of experimental data which serve to characterize the response of otherwise normal living organisms to graded degrees of both respiratory and metabolic acid-base disturbances. A typical nomogram of this type was published recently by Arbus [1][1]. In order for such nomograms to be used appropriately as a clinical aid, the delimited zones must be based upon steady-state observations in individuals known to have uncomplicated acid-base disturbances. Moreover, to the extent that animal data are utilized for such purposes, it must be clearly understood that extrapolation to human beings is tentative and speculative. Although the applicability of this type of nomogram to the clinical evaluation of acid-base disturbances has been widely acknowledged, care must be taken to avoid over interpretation and over simplification. For example, a common misconception is that values that fall within the zone ("confidence band") of a given uncomplicated acid-base disturbance necessarily indicate the presence of an uncomplicated disturbance. In fact, it can be shown that the co-existence of dual or triple ("mixed") disturbances may cause acid-base parameters to fall well within a given confidence band [2].

[1]Figures in brackets indicate literature references at the end of this paper.

For this and other reasons, it must be emphasized that such graphic aids cannot replace the intelligent synthesis of all relevant clinical and laboratory data in achieving a rational diagnosis and therapeutic plan.

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A PHYSIOLOGICAL APPROACH TO ACID-BASE DIAGNOSTICS

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The organism may be conceived as a pluricompartmental buffer solution through which titratable acid and base, because of a relatively high buffer value (β) and the presence of specific control mechanisms, are transported at varying rates at a nearly constant extracellular pH. Correspondingly, the clinical acid-base physiologist is concerned with *acid* and *base* rather than with *acids* and *bases*--being more often than not in no position to imply specific molecular moieties: his principal task is to evaluate extent and control of *hydrogen ion donation* in the body, *i.e.*, to assess sources of gain and loss, fluxes, distribution, concentrations, turnover, and exchange of titratable acid or base. The acid-base physiologist, then, is primarily concerned with the three key variables of buffer chemistry: pH, β, and titratable acid (TA). In terms of Van Slyke's definition [1],[1]

$$pH = -\int \beta^{-1} dTA + pH_0 \qquad (1)$$

where pH_0 is the titration end-point.

Amounts of hydrogen ion transfer taking place under circumstances defined by end-point specification are stated in terms of the acid-base chemical quantity, TA. Magnitudes of that quantity are expressed as the product of a dimensionless number (numeric value) and a unit. The basic unit concerned is (milli) mole of titratable hydrogen ion, conventionally named (milli) *equivalent*. The use of this unit makes it possible to describe the total titratable acidity of any known solution as the algebraic sum of fractional titratable values contributed by individual specific Brønsted acids (and bases) making up the system. Although the milli-equivalent (meq) is being abandoned in current clinical chemical usage [2], it remains particularly convenient for the buffer chemist. The meq is derived from the mmol by qualification with respect to component (H^+) and expressed by multiplying the basic unit by a dimensionless quantity, N, the value of which depends on the acid properties (strength) of the molecular moiety concerned. For a given nonionic acid, meq $\equiv$ mmol titratable H^+ = N·mmol of acid. Accordingly, TA (meq) = C·N·mmol of acid where C is the numerical factor of the millimolar concentration. N is the average number of negative charges conferred per molecule of the nonionic conjugate acid at the neutral pH (pH_0). In general, N is equal to

$$\frac{10^{pH_0-pK_1} + 2\cdot 10^{2pH_0-pK_1-pK_2} \ldots + i\cdot 10^{i\cdot pH_0-pK_1 \ldots -pK_i}}{1 + 10^{pH_0-pK_1} + 10^{2pH_0-pK_1-pK_2} \ldots + 10^{i\cdot pH_0-pK_1 \ldots -pK_i}}$$

[1] Figures in brackets indicate literature references at the end of this paper.

where pK_i is the pK_a value corresponding to the i'th dissociation step of a pluribasic acid. For an arbitrary solution of an acid and its salt(s), we get

$$TA(meq) = N \cdot C_{total} - [B^{n-1}] - 2[B^{n-2}] \ldots . i[B^{n-i}]$$

where C_{total} is the millimolar buffer total concentration and B^{n-x} are conjugate bases of the acid HB^n. At very low or very high levels of pH, the equation must be extended by the terms $+[H^+]$ and $-[OH^-]$.

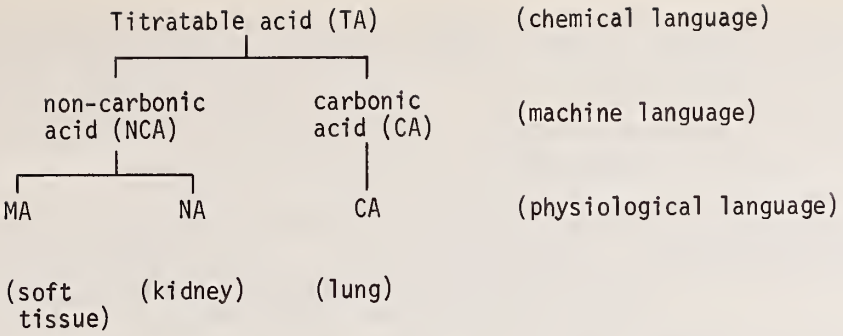
To the extent that different categories of Brønsted acids and bases occurring in the body exibit physiological specificity in the sense that they conform with characteristic patterns of origin, transport, and elimination, the acid-base physiologist must evaluate titratable contributions by such categories separately. Therefore, a very fundamental question is this: Do we know categories of acids and bases the unspecific net titratable contributions by which are subject to organ-specific control and elimination? Viewing the organism as a "pH-stat" which within limits of the capacities of the organ systems involved ensures constancy of the extracellular pH, we may rephrase the question: Do we know categories of titratable acid and base which are subject to independent control in relation to a common extracellular pH?

Whereas the total TA of the body fluids (as determined by closed system titration) is of limited physiological interest, the unique role of *carbonic acid* in acid-base metabolism was early recognized; and the development by Van Slyke [3,4] of methods suitable for CO_2 measurement in biological fluids heralded fifty years of acid-base physiological research. However, as an immediate consequence of the introduction of quantitative CO_2 measurements in biological chemistry, the operational concept of *non-carbonic* (non-volatile) *acid* arose which has been a constant source of controversy. By means of various modifications of the CO_2 technique, clinical physiologists and clinical chemists have endeavored to express--in semiquantitative machine language: "CO_2 capacity" [5], "CO_2 combining power" [6], "alkaline reserve capacity" [7], "standard bicarbonate" [8], etc.--concentrations of *non*-carbonic acid without ever questioning the relevance of this concept. It is characteristic of the situation that when Astrup, *et al.* [9,10] finally succeeded in measuring (by CO_2 titration) exact concentrations of titratable non-carbonic acid in blood, an extensive debate evolved which did not establish any such relevance [11-18]. The difficulties were compounded by a persistent demand by clinicians for *diagnostically* (physiologically) specific acid-base measurements.

Measured concentrations of non-carbonic acid (base) in biological fluids include: (1) titratable equivalents originating in reversible processes of intermediary metabolism or represented by exogenous (dietary) metabolizable "organic" acid, and (2) titratable equivalents originating in irreversible reactions or gastro-intestinal absorption of non-metabolizable acid and base. The *kidney* contributes to acid-base homeostasis by exclusively influencing the extracellular concentration of acid (base) belonging to the second category which in turn cannot be changed by extrarenal mechanisms unless the capacity of the kidney in this respect is overcharged. Correspondingly, changes in the level of renal function cannot *per se* bring about variations in extracellular concentrations of carbon dioxide or metabolically reversible (eliminable) equivalents which depend on *pulmonary function* and *metabolic processes*, respectively. Physiological coupling of variations in extracellular concentrations of titratable carbonic acid ($CA = 0.9522 \cdot [CO_2]_{total}$) and metabolizable and non-metabolizable, non-carbonic acid takes place by the operation of renal, metabolic, and respiratory control mechanisms in relation to a common "set-point" pH. For example, a primary rise in the extracellular concentration of non-metabolizable base will elicit a *corrective* response by the kidney and *compensatory* responses by the respiratory centers [18a] *and* soft tissues [19-22].

1. Definitions

The chemical concept of non-carbonic acid is devoid of physiological specificity: implicit in current knowledge exist three rather than two relevant categories of (titratable) acid and base [22],



where MA, NA ("*net acid*"), and CA represent titratable values of metabolizable acid, non-metabolizable, non-carbonic acid, and carbonic acid, respectively. Each of these three categories of titratable acid may be given an explicit operational definition:

Net acid (NA) ≡ titratable acid on titration to an end-point at pH 7.40, temperature, 37 °C, ionic strength of normal blood plasma, and CA = MA = zero meq/ℓ.

Metabolizable acid (MA) ≡ titratable acid on titration to an end-point at pH 7.40, temperature 37 °C, ionic strength of normal plasma, and NA = CA = zero meg/ℓ.

Carbonic acid (CA) ≡ titratable acid on titration to an end-point at pH 7.40 temperature 37 °C, ionic strength of normal plasma, and NA = MA = zero meq/ℓ.

Obviously primary definitions are required for two of these quantities. Carbonic acid is quantitatively defined as the titratable value of the stoichiometrical CO_2 content/concentration of a given system. Disregarding carbamination and "CO_2 fixation" we have (pK_1 = 6.11).

$$1.0502 \cdot CA = (CO_2)_{total} = CO_2 + H_2CO_3 + HCO_3^- + CO_3^=$$

where CA is equal to the millimolar content of bicarbonate plus twice the millimolar content of carbonate at pH 7.40. Also, for each system or organism concerned, a decision must be made with respect to the distinction between *reversible* and *non-reversible* equivalents.

2. Methodology

Concentration of CA are measured *a.m.* Van Slyke or by the equilibration method [10]. Concentrations of NA may be determined by direct titration following *in vitro* ashing [23,24]. This method, however, assumes an identity between (the extent of) *in vitro* and *in vivo* processes of oxidation which does not always obtain, particularly not in the growing organism where neutral sulfur is stored in new protein. A more convenient approach, which has the further advantages of allowing separation of "addition disturbances" from "subtraction disturbances" and of statements of titratable values at specified levels of ionic strength, is based on the principle of electroneutrality. Thus, for any sample the concentration of NA is given by

$$NA = \Sigma K_a[An^-]_{nm} - \Sigma K_c[Cat^+]_{nm} \qquad (2)$$

where [] are stoichiometrical millimolar concentrations. K_a and K_c denote the average number of negative and positive charges, respectively, per molecule at pH 7.40; and the subscript "nm" signifies non-metabolizable ionic species (in biological fluids largely Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, $SO_4^=$, and phosphate). Accordingly, the *balance of net acid* [22,25-31] is given by

$$NAB = 1.8 \cdot B_P + B_{Cl^-} - B_{Na^+} - B_{K^+} - 2 \cdot B_{Ca^{++}} - 2 \cdot B_{Mg^{++}} \qquad (3)$$

where "B" are millimolar balances representing differences between oral and parenteral intake and renal and gastrointestinal losses. It will be noted that eq. (3) assumes identity between endogenous production (gain) and renal excretion (loss) of sulfate ion. It is apparent that the concept of NAB is deeply embodied in the older medical acid-base literature [30] and that no genuine discrepancy exists between the older "anion-cation terminology" and the acid-base chemical concepts of "acid" and "TA".

Finally, MA is obtained as the difference between NCA, determined by titration at a P_{CO_2} of zero mm Hg, and NA.

3. Normal Acid-Base Metabolism

As a result of endogenous metabolic activity, very large quantities of CA and MA are subject to exchange and cyclic turnover, respectively, at an extracellular pool size (CA + MA) of about 525 meq in the normal adult. Because the pH of extracellular water is approximately 7.42, an extracellular pool of *net base* of the same magnitude must be maintained. The kidney accomplishes this by continuously releasing hydroxyl ions (accompanied by non-metabolizable cation, *i.e.*, net base) to the peritubular venous return at a controlled rate, in the adult about 6300 meq/24 hours. Because in the physiological range of pH free hydroxyl ions exist only in negligible concentrations, OH^- ions generated by the renal tubules are instantaneously and *reversibly* neutralized by endogenous CA and MA. Extracellular net base, therefore, is represented by bicarbonate *and* "metabolizable organic anion" (MOA^-). Of the net base concentration of normal plasma (41 meq/ℓ), approximately 17 meq/ℓ or 41 percent are represented by MOA^- (partly protein). Net base absorbed from the gastrointestinal tract and net acid (sulfuric acid, uric acid, bilirubin, etc.) and net base (urea, creatinine, etc.) originating in *irreversible* metabolic reactions as well as net base communicated by bone contribute to the extracellular pool. The net effect of such processes is normally given by the rate of renal acid *excretion*, equal to the difference between the rates of tubular net base generation and glomerular net base loss [22].

> Urinary net acid is partly represented by the *metabolizable cation*, NH_4^+. By generating ammonia, the kidney does *not* ultimately remove hydrogen ions from the body fluids. It does, however, replace extracellular NA by (somewhat larger quantities of) MA which can be eliminated by extrarenal mechanisms.

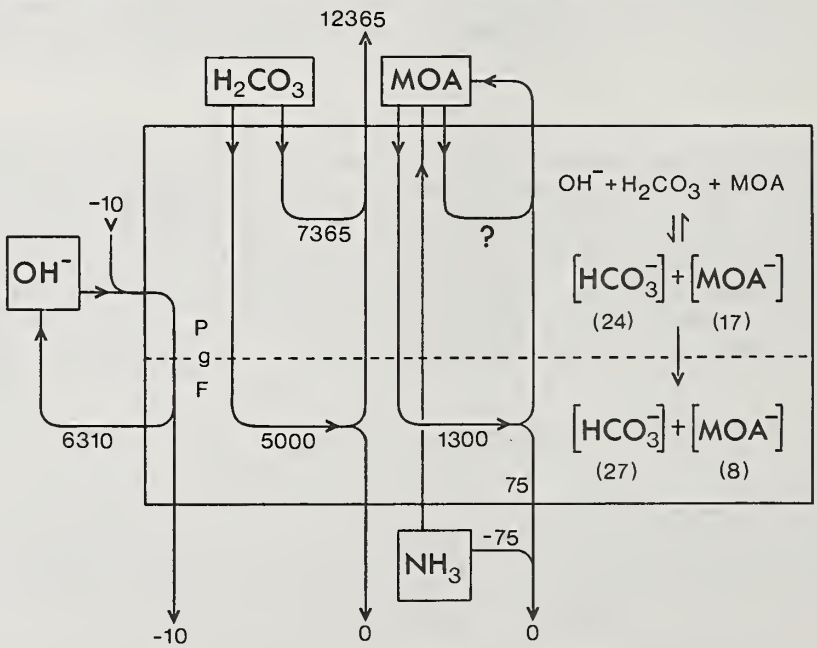


Figure 1. A quantitative outline of acid-base metabolism. P = plasma compartment, g = glomerular membrane, F = glomerular ultrafiltrate.

The acid-base homeostatic "capacity" of the kidney is reflected by the fact that renal net base *exchange* (-20 - 0 meq/24 hours) amounts to only a fraction of a percent of the rate of renal net base *turnover*. An outline of the gross quantitative aspects of acid-base metabolism is shown in figure 1.

4. Acid-Base Status: Clinical Evaluation

The term "acid-base status" implies a set of relevant variables which adequately characterize the acid-base composition of a given sample medium. The acid-base status of biological fluids (blood, plasma, urine, cerebrospinal fluid, milk, gastric juice, etc.) should always be expressed in the *same* chemically unambiguous terms,

$$\left.\begin{matrix} CA \\ NA \\ MA \end{matrix}\right\} TA \overset{\beta}{\Rightarrow} pH = pK_a + \log \frac{[B]}{[A]} \qquad (4)$$

where the right-hand equation refers to selected relevant acid-base pairs, usually the HCO_3^-/H_2CO_3 system. Representative values for some biological fluids are listed in table I. The relationship between the quantities recommended here and some commonly used *plasma* acid-base variables is given by

$$TA - CA = NCA = NA + MA = NBB - BB - K = -BE - K \qquad (5)$$

where NCA is titratable non-carbonic acid, NBB is "normal buffer base" [10], BB is "buffer base" [32], BE is "base excess" [10], and K is a constant, approximately 23.8 meq/ℓ.

Note: If 25 mmol of CO_2 (pK_1 6.11), 17.2 mmol of lactic acid (or 103 g of albumin, TA_{alb} = 0.167 meq/g) and 41 mmol of sodium hydroxide are added to one liter of water in a closed system, the resulting solution will have a pH of 7.40, a P_{CO_2} of 40 mm Hg, and a BE of zero meq/ℓ.

Table 1. Representative values for acid-base status of some biological fluids.

	CA	MA	NA	TA	pH
			(meq/L)		
Blood plasma[a]	24.0	17.0	-41.0	0	7.4
Whole blood[a]	21.7	1.0	-17.4	5.3	7.2[c]
Normal acid urine	1.2	0[b]	5	6.2	5.8
Human milk	4.9	25.0	-24.0	5.9	6.6
Cow's milk	4.1	66.0	-60.0	10.1	6.5

[a] arterialized whole blood, hemoglobin concentration 15 g/100 ml

[b] filtered metabolizable acid minus secreted metabolizable base (NH_3): 50 - 50 = 0 meq/l

[c] hemolyzed blood

The pH-stat concept implies three mutually non-exclusive causes of deviations in extracellular pH, applicable to each regulatory organ system involved: set-point deviation, functional errors, and overcharging by excessive loads. Because of the complexity of the biologic pH-stat careful operational definitions of these are mandatory. By *overcharging* we may understand loading with acid or base, exogenous with respect to the regulatory organ considered and sufficient to produce a stable change in extracellular pH. *Malfunction* may be taken to imply intrinsic disease of the organ concerned; and--by exclusion--*deviations in set-point* may be indicated by functional changes in the absence of (local) disease and acid-base loading. In such terms, diabetic keto-acidosis represents malfunction of soft tissues and overcharging of kidney and lung; obstructive or restrictive lung disease with chronic hypercapnia and a normal extracellular pH represents malfunction of the brain-lung system; and steriod alkalosis and acute salicylism involve set-point deviations for kidney and lung, respectively.

Any such combination of *causes* may lead to a primary change in the TA of extracellular water, the associated change in pH setting off secondary processes of *correction* and *compensation*. The resulting ΔTA is the algebraic sum of concurrent changes in the concentrations of CA, MA, and NA. Each such change is *brought about* by a combination of processes of retention and redistribution and changes in the volume of solvent, due in turn to osmotic redistribution of water across the cell membrane or to changes in water balance. Balance changes may be accounted for by identifying several sources of input and output. This approach to systematic evaluation of clinical acid-base disturbances is shown schematically in figure 2. As an example, figure 3 shows the suggested steps in the

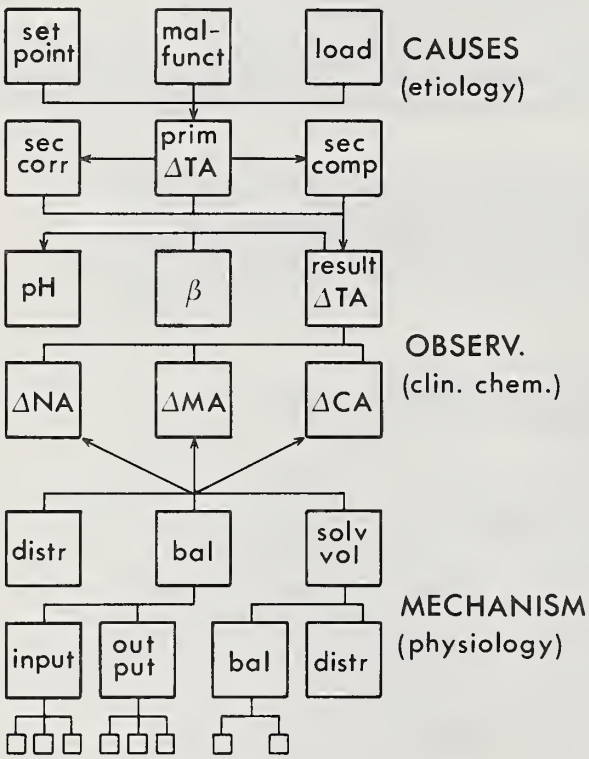


Figure 2. Interpretative steps in the evaluation of the acid-base status.

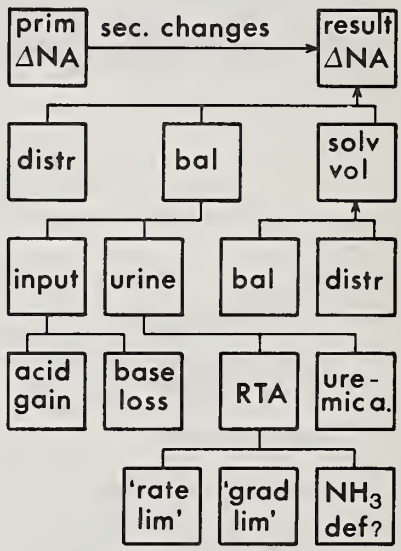


Figure 3. Evaluation of a primary rise in the concentration of extracellular net acid.

diagnostic interpretation of an observed rise in extracellular NA, which on the basis of the pattern of the acid-base status of blood, the total clinical situation of the patient, and existing physiological knowledge is considered to reflect *primary* accumulation of NA in the extracellular compartment. It may be pointed out that a normal acid-base status of

blood does not preclude a significant disturbance of acid-base metabolism. For example, in hyperparathyroidism primary redistribution of body net base (release of skeletal base) may lead to negative net base balance in the absence of significant change in the acid-base status. Because a primary rise in extracellular NA rarely if ever is brought about by processes of redistribution, such changes may be evaluated in terms of the balance of net acid and changes in the volume of solvent ("contraction alkalosis" and "dilution acidosis", respectively) [33,34]. Contraction alkalosis due to negative water balance and a secondary readjustment of the renal tubular set-point contributes significantly to the acid-base status in "gastric alkalosis" [25]; and extracellular dilution acidosis due to osmotic redistribution of water across the cell membrane can be produced experimentally by infusion of hypertonic solutions of aprotes such as sodium chloride and glucose [35]. A positive balance of net acid is a frequent cause of primary increases in extracellular NA. Defining the net acid output as urinary net acid [22,31], and output disturbance leading to NA accumulation is by definition a renal acidosis of which two types are generally recognized: the acidosis of uremia and renal tubular acidosis (RTA). Renal tubular acidosis in turn may be due to failure to establish an adequate trans-tubular pH gradient ("distal" or "gradient limited" RTA [36]), to a relatively low rate of (proximal) tubular net base generation ("proximal" or "rate limited" RTA [37]), or possibly to impaired ammonia excretion [38]. Finally, input disturbances involve extrarenal sources of net acid, in particular oral intake of NA ("addition acidosis") and gastro-intestinal losses of net base ("subtraction acidosis").

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CO_2 SOLUBILITY, pK' AND RELATED FACTORS IN ACID-BASE BALANCE

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1. The Role of pK', CO_2 Solubility, and Temperature in Acid-Base Measurement

An appreciation of the problems of pK' and CO_2 solubility necessitates an understanding of some of the features of the Henderson-Hasselbalch equation that are not always emphasized. With this in mind, a brief restatement of this equilibrium reaction, which provides a system for the examination of acid-base balance, is made here. The thermodynamic equation may be derived from the equilibrium reaction, starting at the partial pressure of CO_2 to the dissociated hydrogen ion activity.

$$PCO_2 \rightleftharpoons \text{dissolved } CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \qquad (1)$$

The first consideration, usually not made a part of the familiar constant, is the solubility of CO_2 itself.

The first step involves the uptake of CO_2 in the plasma. The constant which is necessary for the understanding of this phenomenon is the solubility coefficient α, (Bunsen's coefficient) [1][1], or "S", the solubility factor [2]. Neither appears in the collected constant (pK'), though it is mathematically possible [3,4]. Some consideration has been given to this, but classically it is excluded from the "overall" constant. The latter, often called the first dissociation constant, or apparent pK', is in reality a collection of the ionization and hydration constants and will be treated later. CO_2 solubility may be given as Bunsen's solubility coefficient α, (ml CO_2/ml plasma), often anglicized as "a", and frequently mistaken for the CO_2 factor "S", which is millimoles of CO_2/mm $HgPCO_2$.

Some use α, "a", and "S" interchangeably, as noted recently [5]. This point should be clarified. The original solubility of 0.541 ml/ml [6] was determined by Bohr in 1905, and has an interesting history. Van Slyke [7], in 1928, restudied the solubility, using apparently four samples: two of ox serum, one of normal human serum, and one of normal human oxylated plasma. He obtained values of 0.509, 0.511, 0.506, and 0.511 ml CO_2/cm^3 for these respective samples. The more familiar figure of 0.510, or "S" = 0.0301, is therefore in reality an average of solubilities on ox and human plasma and serum with a water content of 0.925 g/cm^3 at 38 °C. Eisenman, *et al.* [8], Danowski and Gilmore [9], and we [10] found different water contents, and used 0.935 in our solubility studies. The recalculation of Van Slyke's data, using a higher water content, interestingly enough yields values almost identical to ours, as referred to in the "Handbook of Physiology" [11], and the *Ad Hoc* Committee on Methodology of the New York Academy of Science in 1966 [12]. This value is "S" = 0.0306.[2]

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[1]Figures in brackets indicate the literature references at the end of this paper.

[2]Van Slyke astutely recognized [7] that the actual value of α or S is irrelevant if it is used with the appropriate pK'. Note that "ml" or "cc" is used exactly as cited in the original papers.

For years, the variation of CO_2 solubility in plasma was assumed to CO_2 parallel that of water. This was found not to be the case (fig. 1) [10,13], and Severinghaus and others made appropriate corrections which were reflected in pK's, especially at the lower temperatures [10,13]. The variations at 37 °C are small by clinical standards, and should not pose any problems.

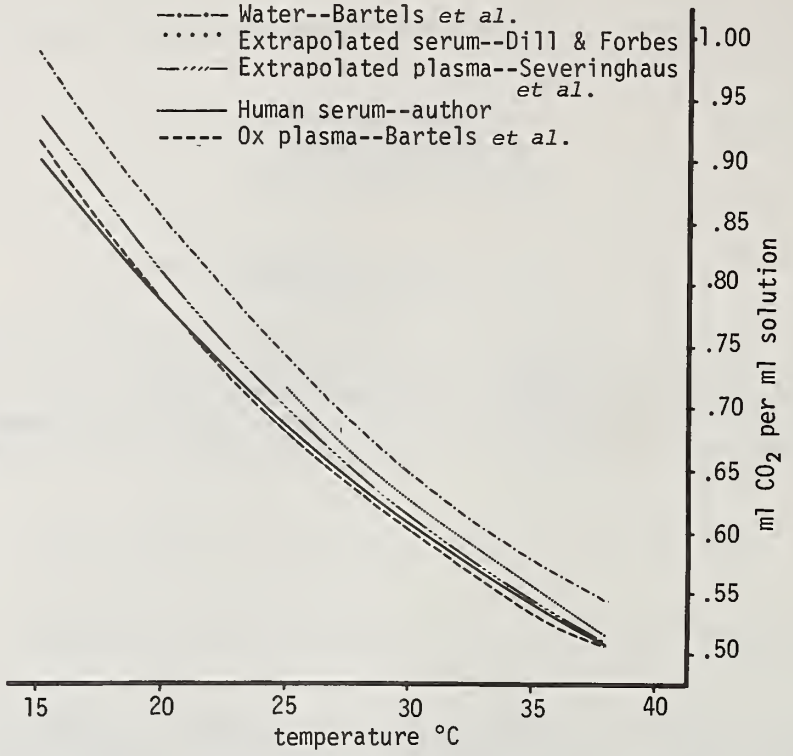

Figure 1. Solubility of carbon dioxide in water, serum and plasma from 15 °C to 38 °C. Solid line indicates CO_2 solubility in plasma does not bear a constant relationship to water (top line), hence to extrapolated values for plasma CO_2 (Severinghaus). (Reproduced, with permission, from the *J. Appl. Physiol.* 19, 893-96, 1964).

Attendant alterations in disease states, blood constituents, viscosity, and ionic strength could conceivably alter CO_2 solubility. These have been considered [14-19], and if the solubility factor is assumed, for the sake of discussion, to be fixed, then these changes, if any, should be reflected in the pK'. This is the constant for the balance of the equilibrium reaction:

$$\text{Dissolved } CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \qquad (2)$$

To interrelate the solubility and these reflected variations in pK', the origin of this constant must be examined. From this "overall" reaction, a pK' may be derived.

The "overall" equilibrium constant falls into two sets of reactions:

1. The Hydration reaction:
   dissolved $CO_2 \rightleftharpoons H_2CO_3$ which may be quantitatively expressed as $(CO_2)K_h = (H_2CO_3)$ with K_h as the hydration constant.

2. The Ionization reaction:
   $H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$ - quantitatively expressed as $(H_2CO_3)K_i = (H^+)(HCO_3^-)$ with K_i as the ionization constant.

These terms may be expressed as concentration or activity. In actual practice, H^+ (or pH) is an activity, since this is what the electrode measures, and CO_2 content (HCO_3^-) is concentration, usually from gasometric measurements. Titration values for HCO_3^- are less commonly used. For this presentation, the practically measured pH (or H^+) will be an activity, and bicarbonate-related entities will be concentrations. Since both dissolved CO_2 and (H^+) + (HCO_3^-) are in equilibrium with (H_2CO_3), the hydration and ionization reactions may be equated, and constants collected.

$$(K_h \times K_i = K'), \text{ resulting in } (H^+) = K' \times (CO_2)/(HCO_3^-) \qquad (3)$$

Although this derived equation is theoretically correct, it does not correspond to the experimental operations involved in the determination of the "overall" equilibrium constant (K' or pK'). One experimentally fixes the CO_2 concentration by equilibrating blood, plasma or serum with a given partial pressure of carbon dioxide gas. This operation fixes the concentration of dissolved carbon dioxide plus the true carbonic acid in proportions determined by the hydration coefficient K_h. This being the case, the numerator "CO_2" of the equation must be CO_2 (dissolved) plus H_2CO_3 (actual), as this is what the analytic determination produces, rather than what the thermodynamic determination alone gives. There are a few bicarbonate ions[3] which arise, but they are insignificant. The "overall" reaction must be rewritten as:

$$(H^+) = K' \cdot (CO_2) + (H_2CO_3) /(HCO_3^-) \qquad (4)$$

Since it has been recognized that this relationship is more properly expressed logarithmically, one may rewrite the equation as:

$$pH = pK' + \log \frac{(HCO_3^-)}{(CO_2) + (H_2CO_3)} \qquad (5)$$

The term $(CO_2) + (H_2CO_3)$ is often referred to as H_2CO_3, creating the false impression that all of the denominator of the Henderson-Hasselbalch equation is carbonic acid as such. The dissolved CO_2 may be potential H_2CO_3, but not actual carbonic acid. This has been a point of confusion to many students of carbonic acid chemistry.

The use of pK', rather than K', has been subjected to the same criticism as the use of pH and H^+ concentration; however, the logic is the same. The most cogent, and sometimes less appreciated, argument has been cited by Davis [20]. Essentially, he notes that a more meaningful description of the equilibria constants is given in their logarithmic form. This is not an accident, nor is it pure convenience nor perpetuation of a misconception. The thermodynamic behavior of a substance is directly related to a potential, the logarithmic function of activity--in this case the log (negative) of the constant K', or pK'.

Davis has expressed it well, "...the most meaningful property describing dissociation equilibria of acids, whether in chemical or physiological systems, is not the dissociation constant itself, but the logarithm of this dissociation constant, pK', which is directly proportional to free energy of dissociation and clearly a measure of the strength of an acid in aqueous solution." Buffering capacity, employing the constant being discussed, is related to the free energy of dissociation, and is not coincidental to the units used [20,21].

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[3]Virtually all of the bicarbonate which appears in actual practice arises from the salt of the weak acid, in this case H_2CO_3. The origin of the bicarbonate does not affect the logic of the derivation; however, the large quantity of these ions from sodium bicarbonate is very important to the buffering capability of the bicarbonate buffer system.

Reasoning in terms of the reactants may be a more appropriate approach. Chemical and physiological activities are more precisely related to the potential, which in turn is a direct function of pH. Therefore, participation of hydrogen ions in physical and physiological reactions is determined by ionic potentials, which are logarithmic expressions of ionic concentrations. (This may be extended to include the other reactants. The Nerst equation, which deals with potentials, applies for similar entities, chemically determined, which react physiologically to the logarithm of molarity.)

Digressing again to the example of pH, which may be more easily visualized, but applicable to pK, one finds hydrogen ion concentration can never really be derived accurately from activity. In theory, there may be pH changes with no H^+ change, if there is an independent change in activity coefficients [20]. pH, hence H^+, is an apparent entity. pH results from emf with an electrode calibrated with buffers of "assigned" values. Though assigned, these values are probably very accurate; however, the pH one gets when blood is measured is relative. The blood or plasma is a biological fluid, differing greatly from the buffer. The pH "number" is obviously only relative. There must be distortion also of the "real" pH through inherent errors in the measurement itself, apart from the buffer. Studies of liquid-junction potentials show this [22], so how, especially in view of the other points, can one arrive at a meaningful H^+ concentration?

The argument of putting acid-base measurement in familiar terms is a pitfall. One takes this entity, calls it a concentration or equivalent, and immediately assumes that the physiologist or clinician can now understand it and can relate this value to other parameters. The end result is like putting a planet and a grain of sand side by side and saying, "Now we are dealing with mass, and all is clear." The student, through working with concentration, has to be constantly aware of the diversity of the magnitude of concentration and, not as has been the case, think of H^+ in the same scope as Na^+ or HCO_3^- concentrations. It is easy to see how the dimensions of H^+ can be thrown out of proportion after thinking of Na^+, HCO_3^-, Cl^-, K^+, etc. It is too easy to lose track of the diminutive nature of hydrogen ion concentration, especially since it is the intensity of acidity that we are seeking. A change in using the inexact concentration concept can only foster this, whereas pH has the dimensionless nature which would diminish this tendency without the need for clarifying that H^+ is not a true concentration, and is calculated or arrived at with the many reservations mentioned above, keeping in mind the magnitude of the quantity.

Also, conversion to concentration does <u>not</u> have the advantage of keeping all of the parameters in the same dimension. In addition to the gigantic difference in magnitude, we are now accustomed to using PCO_2, a far more easily acceptable entity than H_2CO_3, which is not without its conceptual problems, (*i.e.*, H_2CO_3 is really dissolved CO_2 and true H_2CO_3--a point of much misunderstanding). It makes no sense to say that H^+ concentration is similar to bicarbonate concentration (HCO_3^-), and then relate these two to a pressure (PCO_2), especially if this is done in the name of clarity. Filley [23] has stated, with references, an excellent analysis of this controversy.

Since it is more useful to use the partial pressure of carbon dioxide (PCO_2) in the equation, the solubility factor must be introduced to make $(CO_2) + (H_2CO_3) = PCO_2$. This gives to the familiar:

$$pH = pK' + \log \frac{(HCO_3^-)}{PCO_2 \cdot S} \qquad (6)$$

or, for pK' determinations made in the studies discussed:

$$pK' = pH - \log \left(\frac{CO_2 \text{ content}}{S \cdot PCO_2} - 1\right) \qquad (7)^4$$

[4] CO_2 content determined gasometrically in most studies cited above.

The original pk"s determined by Severinghaus in 1956 [16] have been widely used. A value for pK' has been determined in many studies, and has been said to be a function of pH [16,24] and temperature [10], and are affected by disease states [17] and biochemical abnormalities [15]. Re-examination of pK' strongly indicates to us that neither pathologic conditions [26] nor abnormal blood constituents [15], exclusive of temperature, alter the pK' in a practical way. In fact, we could not find significant variations with pH within the clinical range [27]. This is actually similar to the classical studies of Severinghaus [16], and is confirmed by Nunn [28]. By significant, we would mean less than 0.01 between 7.10 and 7.70 (fig. 2). This is not to say that electrodes and other factors described by Maas [4],

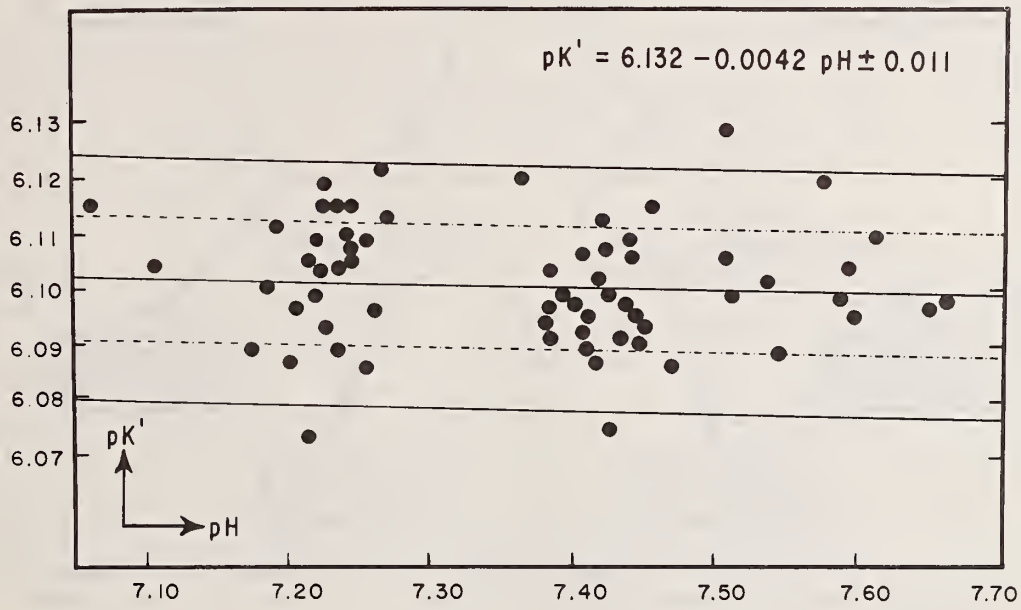

Figure 2. pK variations with pH in human blood. (Reproduced, with permission, from the Archives of Internal Medicine, 126, 699 1970).

Severinghaus [16], and Siggaard-Andersen [24] do not give rise to pH/pK variations, but that for all practical purposes, a pK' of 6.10 on whole blood pH (not corrected for "suspension", liquid-junction effect) plasma or serum CO_2 content, gasometrically determined, and fixed, tonometered CO_2 pressures, is suitable for clinical, and even research, purposes at 37 °C [27]. It is of interest that pK' determinations made using the CO_2 electrode revealed the same pK', but with a much wider standard deviation. The pK's are termed "operational", since no attempt is made to estimate or calculate pHs of plasma because only precise values for whole blood pH were known, and "correction" could not be satisfactorily made despite estimated differences of 0.01 by some [2]. CO_2 content may be determined accurately by the Van Slyke method, though all of the CO_2 is not from HCO_3 or dissolved CO_2. Carbonate and carbamate certainly figure into the picture, but are of questionable significance in the overall picture.

The PCO_2 electrode, although not used as the primary method of classical assessment of partial pressure of CO_2 for pK' work, has shown limitations which, though small, might be considered by some to be important.

The magnitude of the variation in PCO_2 measured by electrode may be seen in an indirect way by comparing pK and S.D., using actual PCO_2 measurements and electrode-measured PCO_2. This reflection in the S.D. of the pK is from ±0.013 to ±0.032 [26]. In terms of actual partial pressure, the standard estimate of the error for actual and electrode PCO_2, measured from approximately 20 mmHg to 50 mmHg, is ±3.5 mmHg. Other studies over a larger range, with 142 samples, give slightly smaller but significant differences of ±2.11 mmHg [29].

Figure 3 breaks down values for pK' as determined in patients and in normal samples by tonometering high and low CO_2 concentrations. The significance is in the similarity. Rigid techniques were observed, as in previous studies [26], and significant differences were not present. This is very consistent with another fifty determinations, using randomly selected patient blood (fig. 4). Whole blood pH and electrode PCO_2 measurements were used with Van

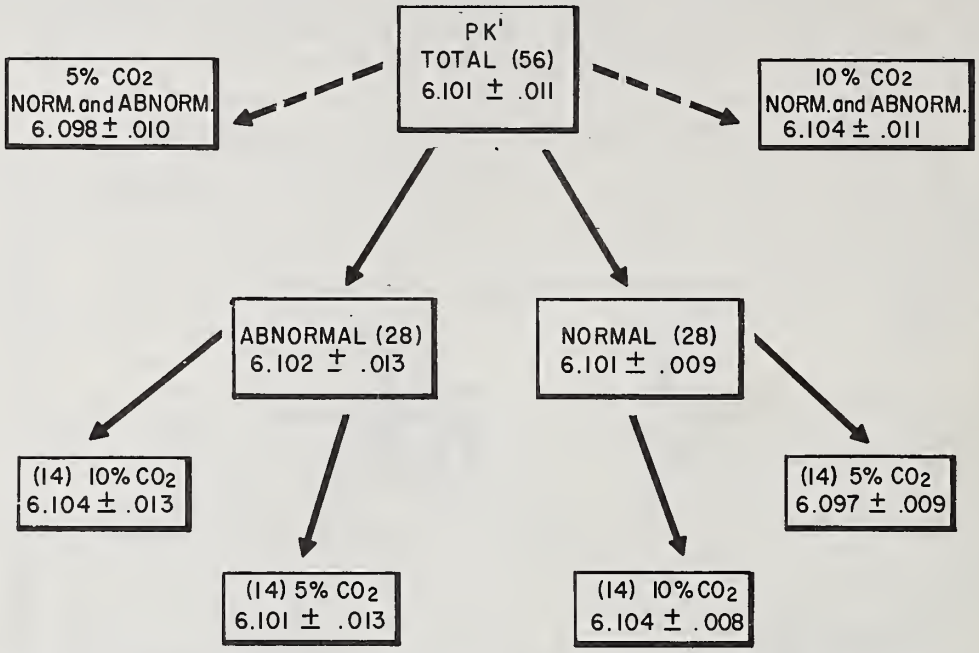

Figure 3. pK's derived from blood of normal and ill patients by tonometry with gas mixtures of approximately 5 percent and 10 percent. No apparent differences noted under various circumstances.

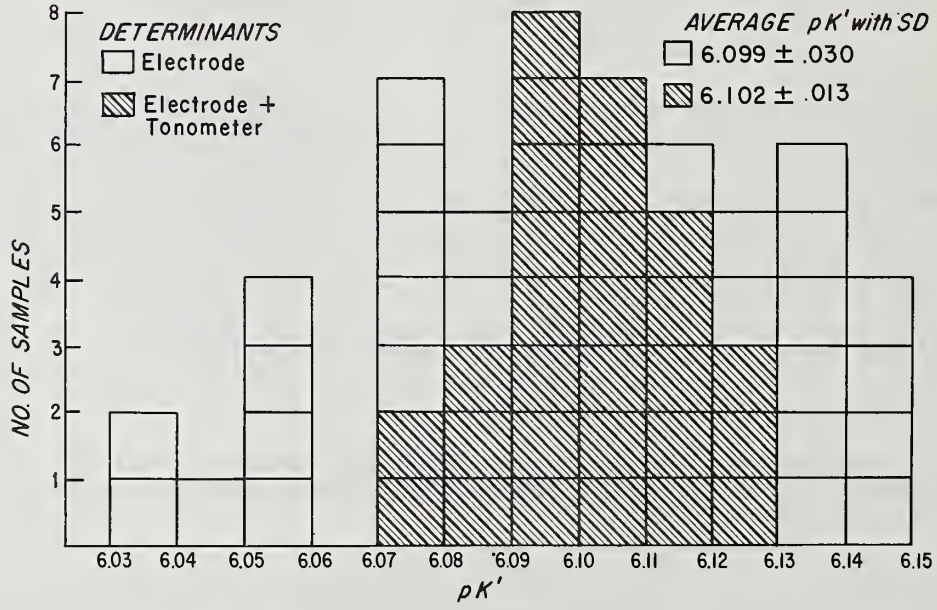

Figure 4. pK' determinations derived from tonometered whole blood with fixed partial pressures of CO_2 and with PCO_2 determined by PCO_2 determined by PCO_2 electrode. Hatched boxes represent two samples, one for each category. Note the narrow S.D. with, the tonometered method.

Slyke CO_2 contents as above. The mean was 6.099. Again, the only difference is in the S.D. of ±0.030, presumably due to CO_2 electrode variations. When twenty-eight of these samples were examined with the tonometry method, the mean was 6.012 and the S.D. was ±0.013.

Perhaps the most cogent point arising from the pK' studies relates to the calculated CO_2 contents' relationship to actual, directly determined Van Slyke CO_2's. This variable (or HCO_3^-) is most frequently used at present, and it is significant in that 54 of 59 samples (most referred to in studies presented here) are within 3 millimoles/liter of the directly determined figure, with the maximum spread of 5 millimoles/liter occurring only once. This is seen with the simple uncorrected pK' of 6.100. Using pK's corrected to pH [10,11] changes the calculated values by less than 1.0 millimole in all instances.

A relatively unexplored, but not totally unrealistic, concept would involve the inclusion of the solubility factor into the pK' itself. Maas [4] has explored this, and Van Slyke [7] himself alluded to this interrelationship many years ago. Simple, logarithmic conversion of "S" (0.0306 to 1.50) added to 6.10 would yield 7.60 as the pK', the only constant in the entire equation. In effect, this is what we actually do (*i.e.*, use an appropriate pK' for a given "S"); however, conceptual and logistical factors make this claim of simplification debatable.

Measurement, by agreement, may be made at 37 °C. Buffers and calibrating gases will not be treated here, but the current practice of using pH/C factors of -0.0148 (0.0150) [30-32] and temperature/PCO_2 factors [33,34] can persist and yield universally acceptable values. Since Nunn [28] and Severinghaus [33] have good evidence of the stability of CO_2 content at different temperatures, this allows determinations of pH and PCO_2 at 37 °C and calculations of CO_2 content (or bicarbonate) which are still valid where pH and PCO_2 are corrected. The other route of correcting pH and PCO_2 to body temperature then, with appropriate solubility factors and pK's calculated to the third variable [11,32] (HCO_3^- or CO_2 content), is also valid but not necessary, and it is cumbersome. Tables [36], slide rules [35], and diagrams [37,38] will simplify the calculations, and whichever is preferred may be acceptable, as long as it yields the same answers for the calculated third variable. This matter, again, is under scrutiny; here standardization should be achievable.

2. Summary

We must use the most refined techniques: stable, sensitive electrodes and electrometers, accurate buffers, good CO_2 electrodes and calibration, and careful sampling and storing--all of which are being discussed, along with the best possible pK's and CO_2 solubilities; however, practicality must prevail. It is our contention that a pK' of 6.100 and an "S" of 0.0306 at 37 °C would not be unreasonable [10-12], and that whole blood pHs be used. Slight variations of pK' with pH would not be unacceptable, but are not essential. The changes in pH with temperatures of -0.015/°C (0.0148) [30,32], as well as PCO_2 ($\Delta \log PCO_2 = F \cdot T$ at 37 °C; F = 0.019) have been well studied and seem to be reasonable standards [28,34].

The misconception which may arise from pK' studies is that the value determined is the appropriate one for the given sample. Because it is the result of two or three determinations, this makes it subject to the accumulation of all the errors inherent in each measurement. This is apparent from electrode and tonometer groups. For example, on one occasion an electrode PCO_2 sample gave a value of 6.030, but 6.102 when determined by tonometry. Another sample gave 6.147 and 6.097, respectively. This suggests to me that there is no "normal" for a given laboratory. pK' may be affected by various factors as noted, but the mean arrived at from the many studies is the operational one for practical use. Considering all the variables, true deviation of the calculated "third factor" is very hard to systematically quantitate, but must be extremely small even using these standard factors outlined here.

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DEFINITION OF OXYGEN SATURATION AND CHARACTERIZATION OF OXYGEN-HEMOGLOBIN AFFINITY

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1. Introduction

The function of hemoglobin is to transport oxygen from the lungs to the tissues. In order to carry out this function optimally, the hemoglobin must take up a maximal amount of oxygen during circulation through the lungs and then yield this oxygen to the tissues perfused during circulation through the body. The ability of the blood to carry out both these functions is determined by the affinity of the hemoglobin for oxygen. Specific definition of oxygen saturation is needed.

The characterization of the relationship between the P_{O_2} and percent oxyhemoglobin saturation--the oxyhemoglobin dissociation curve--has been found to be increasingly complex. It is now known that there are a number of factors which affect this relationship, resulting in shifts in the oxyhemoglobin dissociation curve. The significance of these shifts centers around the fact that the amount of oxygen available to tissues at a given P_{O_2} is changed.

Factors known to affect the oxyhemoglobin dissociation curve are pH, temperature, P_{CO_2} and 2,3-DPG (fig. 1). Increase in pH or decrease in the value of any of the other factors shifts the dissociation curve to the left [1,2][1]. When all these have a "normal value" the oxyhemoglobin dissociation curve has a P_{50} (P_{O_2} for 50 percent saturation of the hemoglobin with oxygen) of 26.7 mm. In addition to the normally observed factors which influence the oxygen-hemoglobin affinity, carbon monoxide, present because of environmental pollution and in cigarette smoke, also effectively shifts the dissociation curve to the left [1,7].

Both acute shifts in P_{50} [3] and what can be considered to be adaptive shifts in P_{50} [4] have been observed.

2. Measurement Techniques

The techniques employed in characterizing oxygen saturation--and more importantly, the hemoglobin-oxygen affinity--of blood must necessarily include measurement of a number of parameters. Direct photometric measurement of oxygen saturation of blood is straightforward

[1]Figures in brackets indicate the literature references at the end of this paper.

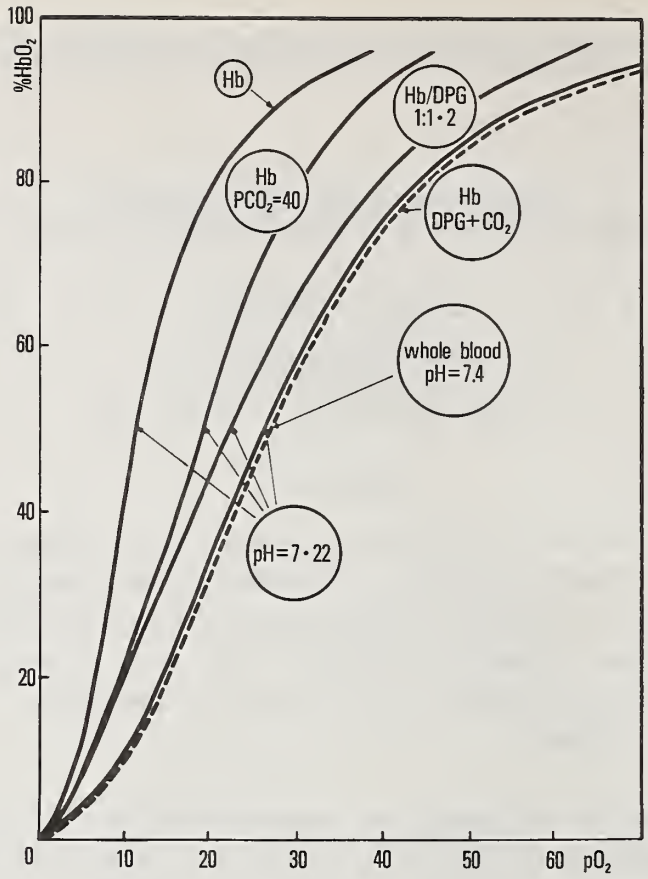

Figure 1. The normal dissociation curve for whole blood at pH 7.4 ---, dissociation curves for hemoglobin solution at pH 7.22 under varying conditions of P_{CO_2} and 2,3-DPG/Hb ratios.

as long as factors such as carboxyhemoglobin and methemoglobin are taken into consideration [5,6]. The typical concentrations of methemoglobin, however, are low enough to not significantly affect accuracy. Failing to characterize the presence of these species can lead to erroneously high values for saturation, however.

Other methods include direct manometric measurement of oxygen content of a particular sample compared with the oxygen content of a sample saturated with oxygen by appropriate means [8]. Oxygen content can also be determined using a fuel cell to consume the oxygen liberated from blood samples, otherwise treated in essentially the same manner as the manometric technique above. Comparing oxygen content with oxygen capacity yields oxygen saturation defined as a percentage of "available hemoglobin"--hemoglobin available for combination with oxygen [9]. Comparing oxygen content with that oxygen capacity theoretically available through measurement of total hemoglobin concentration yields an oxygen saturation defined as a percentage of total theoretical oxygen capacity [6]. The molecular

weight of hemoglobin has been accepted as 64,458 [10]. Each molecule of hemoglobin combines with four molecules of oxygen. Therefore, in traditional terminology, 1.39 ml of oxygen at STP will combine with 1 gram of hemoglobin. Hemoglobin concentration expressed in grams (g/100 ml) multiplied by 1.39 ml of oxygen yields the theoretical oxygen capacity of the blood sample. The relationship in more acceptable terms is 4 moles of oxygen per mole of hemoglobin. Hemoglobin concentration of 2.327 millimoles (15g%) theoretically binds 9.308 millimoles of oxygen (20.85 ml) when complete saturation is achieved.

3. P_{50} Nomogram

Use of measurements typically employed in blood gas analysis, *i.e.*, pH, P_{O_2} and P_{CO_2} combined with the measurement of 2,3-DPG will also yield measurement of oxygen saturation which is accurate [11].

For normal hemoglobins the influence of pH on the oxyhemoglobin dissociation curve has been well characterized and has been reconfirmed; the effect of P_{CO_2} at fixed pH has been established. The characterization of oxygen hemoglobin affinity when changing the ratio between 2,3-DPG and hemoglobin concentrations has been completed as well (fig. 2). Availability of all of these relationships has enabled construction of an empirical nomogram which predicts oxygen saturation which results at 37 °C. This nomogram relates the logarithm of the P_{O_2} required to give 50 percent saturation to changes in pH, P_{CO_2}, and 2,3-DPG/Hb ratio (fig. 3). The nomogram can be used to calculate any of the parameters when the other three are known, and could be used as a means of indirectly measuring 2,3-DPG if that were of interest.

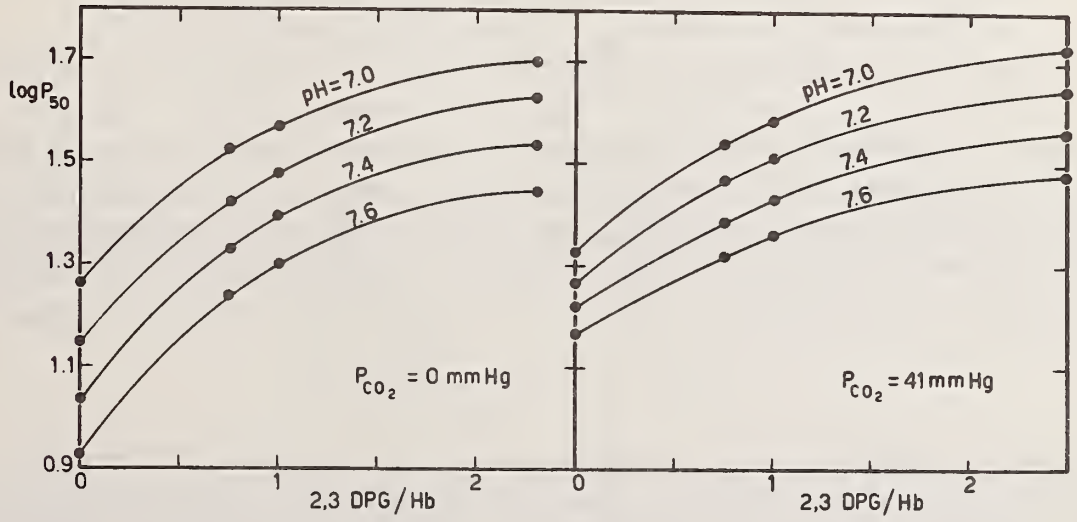

Figure 2. Values of log P_{50} of human blood *versus* 2,3 DPG/Hb ratio at zero P_{CO_2} and P_{CO_2} = 41 mmHg.

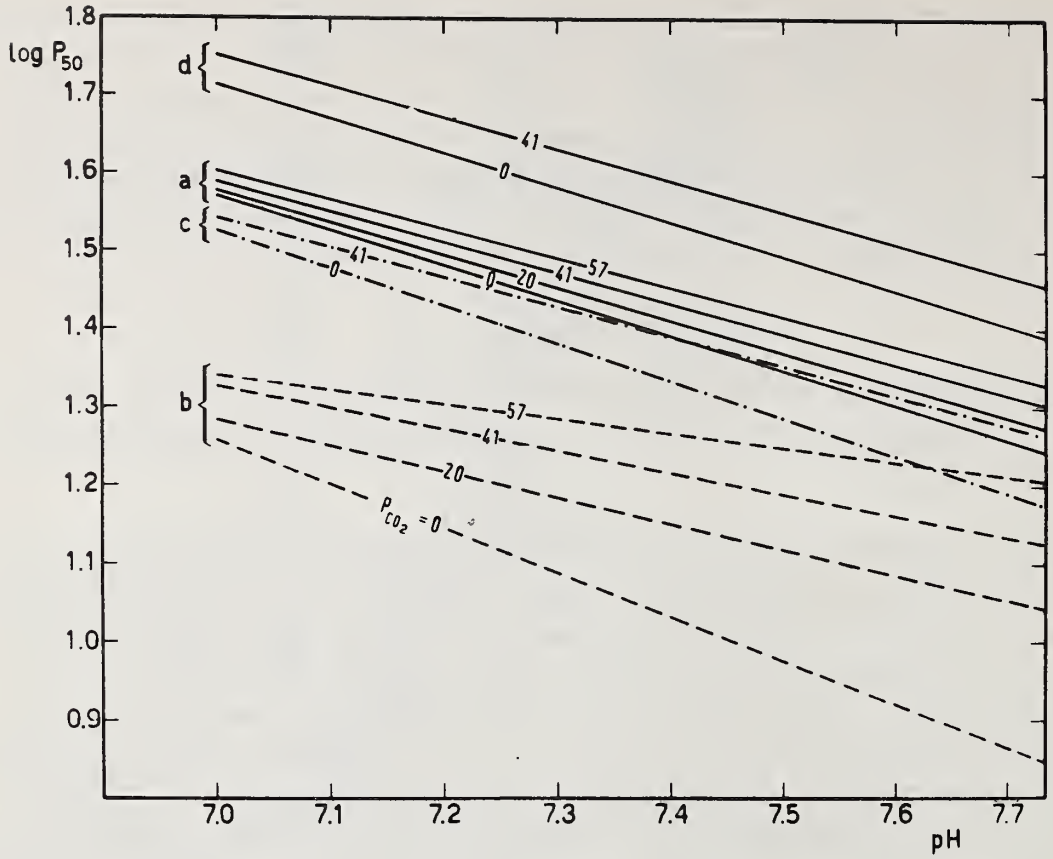

Figure 3. Values of log P_{50} as a function of pH and P_{CO_2}: a) normal human blood at 37 °C; b) human blood with no 2,3 -DPG; c) human blood with 2,3-DPG/Hb ratio = 0.75; d) human blood at P_{CO_2} = 0 and 2,3-DPG/Hb ratio = 2.3 and P_{CO_2} = 41 mmHg and 2,3-DPG/Hb ratio = 2.5.

The work which led to the data which supports this nomogram was carried out by a group comprised of researchers from Instrumentation Laboratory, Inc., the University of Milan and University of California at Santa Barbara. The aim of the work was to extend studies which have been performed on the effects of these hemoglobin effecters from diluted and purified hemoglobin solutions to whole human blood under near physiological conditions of temperature, P_{CO_2}, pH, etc.

The oxygen dissociation curve (ODC) for a concentrated (*ca.* 25 percent) sample of human hemoglobin in 0.1 *M* KCl serves as a basis for determining the effect of the individual species. These ODC's were obtained using the "open" tonometric technique at a constant pH of 7.22. This pH was chosen since it has been demonstrated [15] that an extracellular pH of 7.4 closely corresponds to an intracellular pH of 7.22. Thus, at the same intracellular pH only two cofactors, CO_2 and 2,3-DPG are required to make the ODC of a hemoglobin solution correspond to the ODC of whole blood.

Table 1. Values of log P_{50} as a function of pH and P_{CO_2} interpolated through the data.

2,3-DPG / Hb	P_{CO_2}	pH: 7.0	7.2	7.4	7.6
	0	1.260	1.148	1.038	0.926
0	20.874	1.285	1.820	1.154	1.088
	41.535	1.328	1.274	1.220	1.166
	57.794	1.342	1.305	1.272	1.232
0.75	0	1.528	1.432	1.336	1.240
	41.535	1.545	1.468	1.396	1.316
	0	1.575	1.484	1.394	1.306
1	20.874	1.584	1.496	1.412	1.330
	41.535	1.592	1.512	1.436	1.356
	57.794	1.604	1.530	1.456	1.380
2.3	0	1.716	1.628	1.540	1.452
2.5	41.535	1.756	1.672	1.592	1.512

The position of the ODC along the P_{O_2} axis will be described in terms of its P_{50} (P_{O_2} at which the hemoglobin is 50 percent saturated with oxygen). Plotting log P_{50} *versus* pH yields straight-line relationships which are a function of P_{CO_2} and 2,3-DPG. Table 1 indicates the values for log P_{50} interpolated from the data generated at varying levels of P_{CO_2} and 2,3-DPG.

These data can be either represented in a nomogram or mathematically derived from an empirical formula. Both the nomogram and the empirical function allow calculation of pH, P_{CO_2}, log P_{50} or 2,3-DPG/Hb ratio when the other three are known. The final monogram (fig. 4) has been completed by adding a scale for the P_{O_2} and a corresponding scale for S_{O_2}, assuming that the relation between P_{O_2} and S_{O_2} conforms to the classical Hill's equation [12].

$$\log \frac{S_{O_2}}{100 - S_{O_2}} = \log K + n \log P_{O_2}$$

At 50 percent saturation log K = -n log P_{50}. The value of n was assumed to be 2.7. Clearly this equation can only be assumed to give a rough estimate of the relation between S_{O_2} and P_{O_2}. Other workers have also found that n varies for normal human blood when CO_2 and 2,3-DPG are varied [13]. With these limitations in mind, it is possible to obtain the function S_{O_2} *vs.* P_{O_2} derived from Hill's equation at a definite value of log K = (-n log P_{50}).

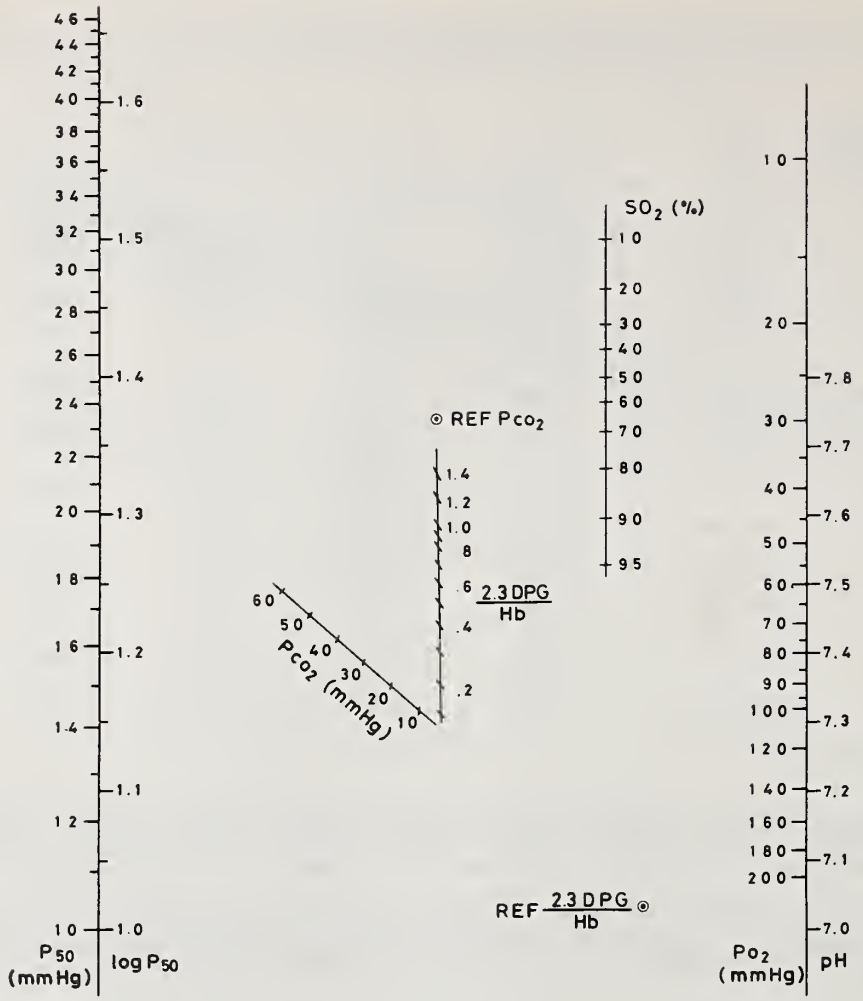

Figure 4. The oxygen dissociation curve and P_{50} nomogram.

Use of the P_{50} nomogram is relatively straightforward (fig. 5). If it is required to find log P_{50} for values of pH = 7.4, P_{CO_2} = 40 mm, G = 1.0 the steps are as follow: (1). Line 1, connecting P_{CO_2} with Ref. P_{CO_2} is traced; 2) G = 1.0 and Ref. 2,3-DPG/Hb are connected by line 2; (3) A straight line connecting pH = 7.4 with the log P_{50} scale is traced through point of intersection of lines 1 and 2. The value of P_{50} = 26.7 mm is read on the appropriate scale. As shown in figure 6, the value of G may be established by knowing P_{50}, P_{CO_2}, and P_{O_2}.

Figure 7 indicates the means by which an ODC may be constructed by knowing P_{50}, P_{CO_2}, and P_{O_2}.

By inserting values of G, pH and P_{CO_2} into the mathematical expression which is expressed below, values of log P_{50} can be directly calculated.

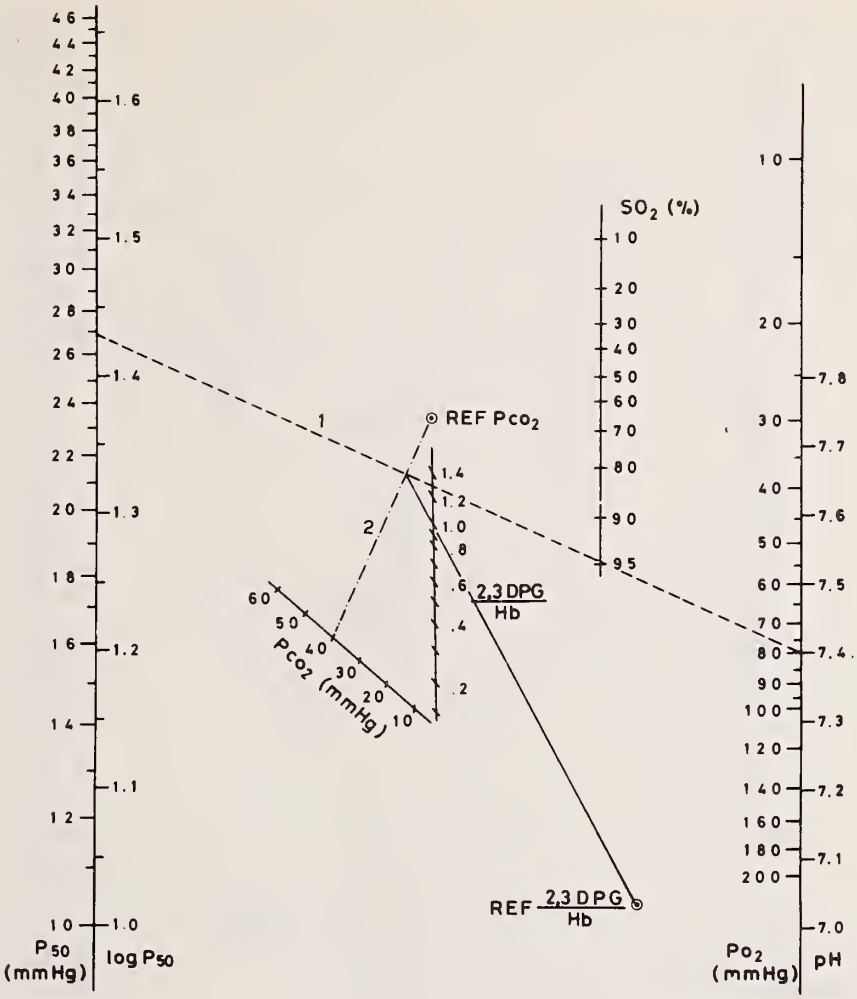

Figure 5. Use of nomogram to determine P_{50} from measurement of 2,3-DPG/Hb ratio, P_{CO_2} and pH (see text).

$$\log P_{50} = 1.9729 \times 10^{-3} G^2 pH\ P_{CO_2} - 0.6002 \times 10^{-3} G\ pH\ P_{CO_2} + 3.9274 \times 10^{-3}$$

$$pH\ P_{CO_2} + 206.5 \times 10^{-3} G^2 pH - 247.79 \times 10^{-3} G\ pH - 410.31 \times 10^{-3}$$

$$pH + 15.85 \times 10^{-3} G^2\ P_{CO_2} + 1.157 \times 10^{-3} G\ P_{CO_2} - 25.948 \times 10^{-3}$$

$$P_{CO_2} - 1.6337\ G^2 + 2.2427\ G + 4.125.$$

Evaluation of errors which will occur in values for P_{50} at pH = 7.0, 7.2, 7.4 and 7.6; P_{CO_2} = 0, 20, 41 and 57; and G = 0, 0.75, 1, 2.3 and 2.5 has been carried out by use

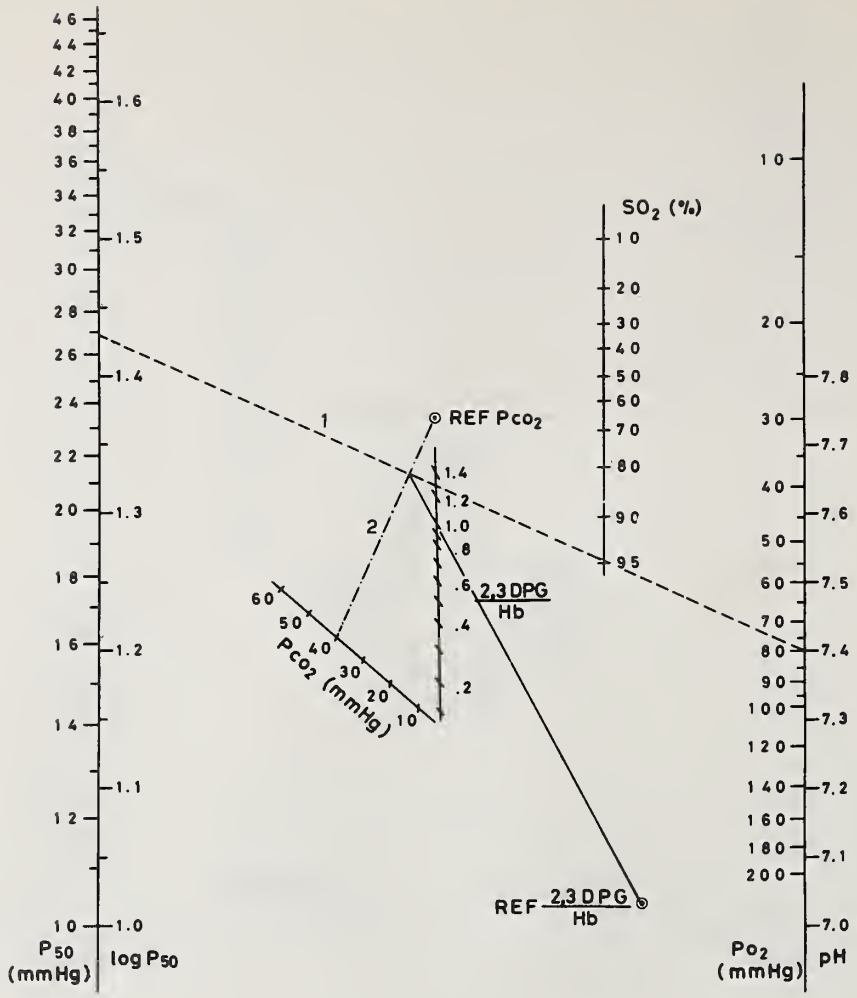

Figure 6. Use of nomogram to determine 2,3-DPG/Hb ratio from measurement of P_{50}, P_{CO_2} and pH (see text).

of the mathematical expression. Table 2 gives the results obtained for log P_{50} using the mathematical expression and can be compared with the results interpolated from the empirical data. In the range of values which encompasses most physiological and pathological conditions, the maximum discrepancy of log P_{50} is 0.037 at a value of log P_{50} = 1.632 which is about 2 percent. In the range of G from 0.1 to 1.0 the maximum deviation is 0.011 for log P_{50} = 1.328. Overall, it is likely that the error in the determination of log P_{50} or G in the range of the variables indicated in the nomogram does not exceed 3 percent of the desired quantity.

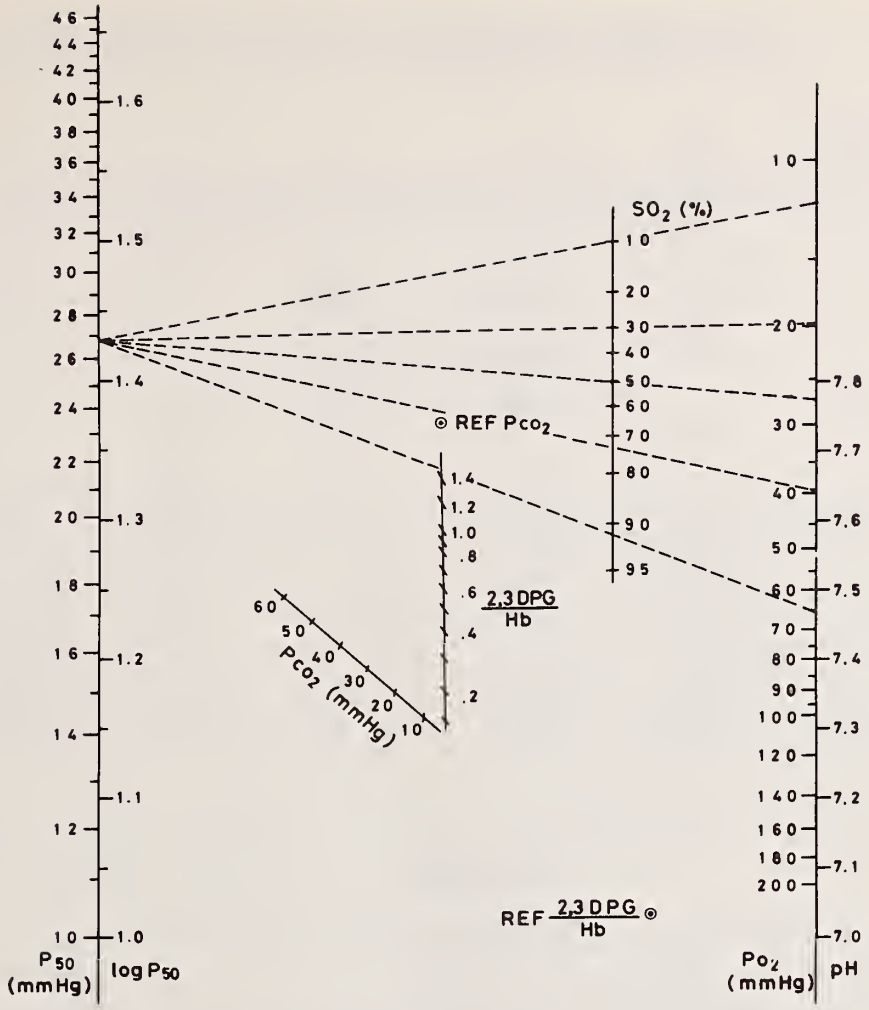

Figure 7. Use of nomogram to construct oxygen dissociation curve from measurement of P_{50} (see text).

This work is by no means final, and use of the nomograms can safely be applied only to blood with normal hemoglobin concentration in the absence of pathological hemoglobins. We have no data available at this time, for instance, on blood from individuals who are anemic. In these individuals G is altered (mainly by decrease in hemoglobin concentration) from its normal value [14].

The purpose here is to demonstrate the requirements for an accurate assessment of oxygen saturation of blood samples. The several species which will affect the relative hemoglobin-oxygen affinity must be accounted for in order to accurately make an assessment.

Direct photometric measurement of oxygen saturation, combined with measurement of blood gas parameters pH, P_{CO_2}, and P_{O_2} yields the required information for correct assessment of hemoglobin-oxygen affinity.

Table 2. Values of the function log P_{50} = f(pH, P_{CO_2}, G) derived from equation.

2,3-DPG / Hb	P_{CO_2}	pH: 7.0	7.2	7.4	7.6
0	0	1.253	1.171	1.089	1.007
	20.87	1.285	1.219	1.154	1.088
	41.53	1.317	1.267	1.218	1.169
	57.79	1.342	1.305	1.269	1.232
0.75	0	1.528	1.432	1.336	1.240
	41.535	1.545	1.469	1.392	1.316
1	0	1.573	1.482	1.392	1.302
	20.87	1.584	1.499	1.415	1.330
	41.53	1.595	1.516	1.437	1.358
	57.79	1.604	1.529	1.455	1.380
2.3	0	1.426	1.448	1.471	1.493
2.5	41.53	1.625	1.595	1.565	1.535

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PROBLEMS ASSOCIATED WITH THE DEFINITION OF MEASURED AND CALCULATED QUANTITIES IN BLOOD pH AND GAS ANALYSIS

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For most students, the subject of acid-base balance has the reputation of being a singularly complex and confusing one. This seems to me to be due in large part to the multitude of methods which have been used and quantities which have been defined to express the acid-base status of an individual. Much confusion has been caused by the existence of several methods, that might all be in common use, and that measure similar, but not exactly the same, quantities. Another obstacle to understanding is the existence of several terms to denote a single quantity, or group of quantities that differ from each other only in a trivial way. Moreover, many modern and widely used textbooks contribute to the problem by devoting a disproportionate amount of space to a discussion of methods and quantities that are generally agreed to be outmoded, and giving inadequate space to a discussion of legitimate differences of opinion or differences in usage which exist in this field today.

My objective in this presentation is to outline some of the problems that exist in the area of definition of quantities used in blood pH and gas analysis. I think it will be clear that many non-trivial questions exist that will not, of course, be answered at this meeting, but that do deserve our careful consideration.

The quantities that perhaps cause the fewest problems, in terms of their meaning, happen to be the three most commonly measured, namely pH, P_{CO_2} and P_{O_2}. This is not to imply that no questions exist in the definition of these. The pH of an unknown solution is defined and determined in an operational way by comparison with the pH of an accepted standard solution. Systematic biases in blood pH measurements between laboratories often occur however, even when both are using the same pH standard. These can be the result from differences in the type of liquid junction, differences in bridge solution composition, different temperatures of the salt bridge, or junction potential differences due to the so-called "cell effect" seen with whole blood. While it would be worthwhile to work toward eliminating such inter-laboratory variations, it must also be remembered that accuracy as a concept in pH measurements with whole blood is much more difficult to deal with than with most other measurements. Quoting from Bates' monograph [1][1],

> "...the experimental pH can never be an exact measure of either the concentration or the activity of hydrogen ion.... It is safe to say that no quantitative interpretation of measured pH values should be attempted unless the medium can be classified as a dilute aqueous solution of simple solutes."

The need, therefore, is to establish a convention for the measurement of pH in blood, so that consistent and reproducible numbers can be obtained in different laboratories and with different instruments.

With regard to the gas tensions, P_{CO_2} and P_{O_2}, definition is not a problem, but again,

[1]Figures in brackets indicate the literature references at the end of this paper.

consistent measurement of these quantities is often a problem. Differences in electrode response time at different gas tensions and differences in measured gas tensions between gas and liquid standards are two common reasons for inconsistent and inaccurate measurements. These problems are actually ones of standardization, and perhaps of instrument specifications, and should be discussed later in this symposium.

Another measurement which is routine in most clinical chemistry laboratories is CO_2 content, but this term has been used in connection with many different methods, not all of which give equivalent results. The determination has been made by measuring the volume of CO_2 released from acidified serum or plasma or measuring the color change in a weakly buffered indicator solution on reaction with an aliquot of this CO_2, for which different indicators and buffers have been used. The measurement can be made with or without the addition of base as a preservative, with or without equilibration of the sample to a known CO_2 tension, and after centrifugation of the blood at either body temperature or room temperature. Clearly, there is a need to adopt a single definition for this quantity, and then to determine the extent of systematic bias that is present in the several methods that are available.

Some other quantities that are often measured directly, relate to the oxygen transport status of an individual. These too can have more than one meaning, depending on the particular method of measurement. Hemoglobin may be measured as oxyhemoglobin alone, or in combination with reduced hemoglobin, carboxyhemoglobin or methemoglobin. Likewise, oxygen saturation, which is actually a derived quantity, can be calculated with or without terms to include the effect of carboxyhemoglobin or methemoglobin. Spectrophotometric methods with measurements at one, two, three or four wavelengths have all been used, and will give different results with the same blood sample if carboxyhemoglobin or methemoglobin is present. To add to the confusion, there is also the very common practice of calculating oxygen saturation from a measured pH and P_{O_2}. The equations or nomograms used for this purpose are constructed from a standardized oxyhemoglobin dissociation curve--not necessarily the same one--which is only valid under a single set of conditions, including CO_2 tension, 2,3-diphosphoglycerate concentration and concentrations of carboxyhemoglobin and methemoglobin. A systematic bias is always present when all these conditions are not met by the specimen of blood being analyzed. Moreover, it is quite common to find that two of these nomograms give significantly different answers, particularly at high oxygen saturations.

Increased interest in the actual oxyhemoglobin dissociation curve which is obtained in a given sample of blood has led to the more common calculation of P_{50}, the oxygen tension which corresponds to 50 percent saturation. Here too, careful definition and consistent conventions are needed in order to avoid inter-laboratory differences. First, the meaning of oxygen saturation must be established, and second, agreement must be reached on the values for constants to be used in the Hill equation, Adair equation, or other relationship used to calculate P_{50}.

A final quantity of interest related to oxygen transport is oxygen content. This may be measured directly by either the manometric Van Slyke method or by a technique in which all the oxygen in the sample is reduced electrochemically. Alternatively, oxygen content can be calculated as (K x total hemoglobin x oxygen saturation). This calculation will be meaningful only if two conditions are met. First, total hemoglobin and oxygen saturation must be expressed in units that are consistent with each other, and second, a value for K must be agreed upon. The theoretical value for K can be calculated from the molecular weight of hemoglobin A and the molar volume of oxygen at standard temperature and pressure, and is equal to 1.39 ml O_2 per gram of hemoglobin. This value is not always considered the best to use however, due to reports of an unexplained systematic bias between calculated and measured oxygen contents. Again, it is clear that a convention needs to be adopted in order to make it possible to obtain values that are meaningful and consistent among laboratories.

Returning to the definition of acid-base status, I have described some problems having to do with measured quantities and I would like to discuss now some factors related to derived quantities. These are sources of confusion and inconsistency, again because a given quantity is often calculated using different definitions or different values for constants. First, consider the quantities that may be calculated using the Henderson-Hasselbalch equation. If we assume that accurate values of pH and P_{CO_2} are available, the Henderson-Hasselbalch equation allows calculation of plasma bicarbonate and CO_2 content. The condition

that must be met in order to insure consistent results among laboratories is, of course, that a single value for the dissociation constant of carbonic acid be adopted and a single number for the solubility coefficient of CO_2 in plasma be used. Careful experimental work has resulted in apparently accurate values for these constants, so this should not present a serious problem.

A more difficult quantity to reach a consensus on in the past has been base excess. This reflects the fact that base excess can be defined in relation to various fluid compartments in the body, including plasma, whole blood, interstitial fluid, or some combination of these. The parameter that is necessary to define base excess mathematically is the buffer capacity of the fluid. Although an early definition of base excess was based on the buffer capacity of whole blood, the most widely accepted definition at present is based on the buffer capacity of extracellular fluid (*i.e.*, whole blood together with interstitial fluid) which has been determined *in vivo* by several groups. Unfortunately, although most workers who are active in the field of blood pH and gas analysis seem to agree on the advantages of the so-called extracellular fluid base excess, several widely used textbooks, published within the last five years, do not mention this quantity at all, but persist in using the older definition. Clearly, when there is indeed consensus among experts, then the conventions that have been agreed upon must be effectively publicized if they are to influence current teaching.

Finally, reference should be made to the many other terms that have been used in the past and in some cases continue to be used to describe various aspects of the acid-base status of an individual. This list includes alkali reserve, bicarbonate reserve, buffer base, buffer anions, CO_2 combining power, acid-base ratio, standard bicarbonate and CO_2 capacity. Some of these terms are redundant; some are simply no longer useful. All of these terms may be found in recently published textbooks. The need for derived quantities can perhaps be placed in perspective by keeping in mind that acid-base status is completely determined if only two quantities are known, for example, pH and P_{CO_2}. The justification for continuing to use other quantities must rest on a real practical advantage in the understanding or treatment of acid-base abnormalities.

In summary, present usage of both measured and derived quantities for blood pH and gas analysis is often inconsistent and confusing. This area of clinical chemistry would benefit greatly from international agreement on the mathematical definition of quantities and on values for appropriate constants. It would then also be necessary for the areas of agreement to be widely publicized. One change which I feel would be a significant improvement would be for scientific journals to adopt uniform criteria for nomenclature and definition of terms in this area. This has been done successfully by other journals in the areas of spectrophotometry and statistics.

The most important reasons for precise definition of terms and agreement on conventions are that they will make possible measurements whose results are reproducible among laboratories, it will greatly facilitate the interpretation and comparison of data generated in different laboratories, and it will make the job of those who apply the results of these measurements to patient care less complex. The desirability of achieving these goals is something that can certainly be immediately agreed upon.

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ARTERIAL AND VENOUS BLOOD SAMPLES IN ACID-BASE BALANCE

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The field of pH and blood gas measurement has stood as the stepchild of anesthesiology, pulmonary medicine, nephrology, endocrinology, and research itself. It has never gained the status of a full-fledged discipline. Because of these multi-faceted relationships, different concepts concerning sampling, measuring, calibrating, reporting, and interpreting have arisen.

A simple example of this is seen in the use of PCO_2 by the respiratory physiologists and pCO_2 by others. In this simple example, the symbol "P" is used to mean pressure, whereas it is also used in the designation of a constant, such as pK'. The respiratory physiologists have achieved some measure of uniformity by agreeing upon symbols and nomenclature for international use. However, "α", the solubility factor (ml CO_2/ml plasma) and "S", the coefficient (mm PCO_2/ml plasma) are still used interchangeably.

This could be logically extended to the entire field of acid-base balance, as it is used in the various disciplines mentioned above. The logical way to achieve consistency and uniformity is to make pH and acid-base balance a specialty in itself. This would be done with the understanding that this area would not create isolation.

In doing this, there must be general agreement in the many areas of this subject. This workshop, I believe, has addressed itself primarily to this point. Many of these areas are being discussed here, and no single paper can encompass the entire field. One area in which there has been debate is that of the site of the sample taken to be measured [1-4][1]. It is obvious that arterial blood is essential when dealing in matters of pulmonary physiology; however, there has been no universal agreement regarding the role of venous blood in the other physiologic areas. Gambino [5-7] has been an outspoken advocate of venous blood, and has used venous blood where pH and PCO_2 have been of primary concern. For others, the addition of PO_2 is essential, and dictates the origin of the sample. I am not aware of any clear definition of the role of venous blood measurements, despite consideration of mixed venous (brachial and hand) blood. Much has been written about "arterializing" venous blood, and using various techniques to ensure the uniformity of venous samples. There is evidence of the similarity of venous and arterial blood when drawn from the brachial vessels under controlled conditions; however, we have encountered some dissimilarities despite rigid sampling and measuring techniques. At times, there may be very little difference, but it has been difficult to determine the conditions which produce A-V dissimilarities.

Under controlled conditions, the PCO_2 of arterial blood will change dramatically, relative to venous PCO_2, with alteration in ventilation. Figure 1 indicates this point. In the studies illustrated here, blood samples were taken from the inferior vena cava and distal aorta in each of eight dogs, before and following a twofold increase in minute ventilation [8]. The dogs were intubated, and had the appropriate vessels cannulated, with a one-hour steady state during which the PCO_2 measured by electrode was kept within ±3-4 mm. The figures show changes which occurred at various intervals in both the arterial and venous systems. The rapidity of the fall of PCO_2 in arterial blood, and the lag in decrement for venous blood illustrates just one facet of this problem. The lowering of the arterial partial pressure of carbon dioxide is consistent with the change in ventilation, and the venous pressures reflect changing carbon dioxide levels at the tissue level.

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[1] Figures in brackets indicate literature references at the end of this paper.

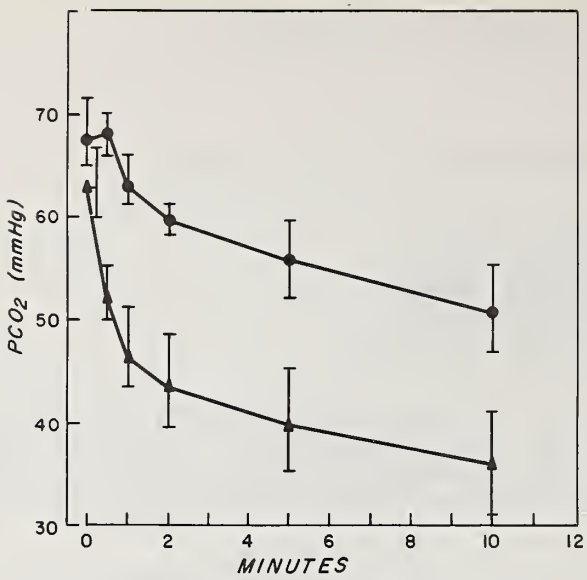

Figure 1. The average and mean changes in venous (●) and arterial (▲) PCO_2 resulting from a two-fold increase in minute ventilation. (Reproduced with permission of *The Journal of the Maine Medical Association.*)

Each value is appropriate for the situation in the unsteady state, and there is no "right" or "wrong" value. There is, then, the logical extension of this simple maneuver to the patient. There is no axiom which states that venous and arterial blood should have a fixed difference in either sample, or will represent one physiologic set of circumstances. The misconception occurs when one tries to correct arterial for venous, or venous for arterial, blood sample values. The major issue is, "which sample of blood reflects the clinical state, and does it provide the information necessary for the appropriate treatment of the patient?"

Table 1 illustrates the A-V differences which were measured in fifty consecutive patients with various disease states. Numbers 32,35,36,37,39,42, and 46 suggest abnormalities if the venous blood is observed. This is not the case. No statistical analysis can be made here, but neither the nature of the illness nor its severity appears to correlate with the differences observed. That there are differences is no surprise to some, but may be challenged by others. Yet the facts must speak for themselves. Although there is no systematic, objective proof of the matter, the arterial values appear most consistent with the patient's clinical state. In those instances where one would not anticipate abnormalities, venous blood measurements suggest that there are. All this is done using the criteria set forth for drawing and measuring pH and blood gases [6]. The reasons for these differences are not apparent, and no obvious technical errors were made in the determinations. The conditions for sampling and measuring have been made elsewhere, and strict adherence to these has been used. Reliability of calculated variables has been stated [1-5]. Transient changes in venous blood flow may account for some of these differences; however, it was impossible to predict when the wide A-V differences would occur. It could be argued that the venous measurements in reality reflect the tissue state, which is perhaps desirable, but this may only be representative of the area from which the sample is taken. Here a mixed venous blood sample would be the logical site, but not practical to obtain.

I have been an advocate of the use of venous blood, but over the years I must concede that I find myself relying more and more on arterial values, even in disease states which are not primarily of respiratory origin. Now technicians can easily obtain samples, which obviates a previous objection, and frequent punctures have not been a problem. The circumstances are such that the calculated CO_2 content, or bicarbonate, made when the pH and PCO_2 are measured, is generally quite similar, or differs by a consistently small amount. This means to me that the Henderson-Hasselbalch equation is giving correct CO_2 contents, or bicarbonates, but that the pH and PCO_2 values may be misleading. On other occasions, abnormal pH's and PCO_2's were found on venous blood, when no disorder existed after subsequent arterial samples were taken. I have become more and more convinced of the reliability of arterial pH and PCO_2 as representative of the severity of the disease state, even though venous CO_2 contents are usually consistent. This appears to apply more to PCO_2 measurements which we have found to be extremely variable in venous blood.

Table 1. A-V blood gas differences in fifty patients.

	pH			PCO_2			TCO_2		
	A	V	Δ	A	V	Δ	A	V	Δ
1.	7.46	7.43	.03	41.5	46.6	5.1	29.6	31.0	1.4
2.	7.41	7.37	.04	43.5	49.0	5.5	27.7	28.5	0.8
3.	7.46	7.43	.03	40.4	44.3	3.9	28.8	30.0	1.2
4.	7.41	7.38	.03	42.0	45.0	3.0	26.7	26.8	0.1
5.	7.44	7.41	.03	36.6	41.5	4.9	25.0	26.4	1.4
6.	7.46	7.43	.03	37.7	41.5	3.8	26.9	27.6	0.7
7.	7.37	7.35	.02	53.4	58.0	4.6	31.1	32.1	1.0
8.	7.46	7.43	.03	29.8	31.3	2.5	21.3	20.8	0.5
9.	7.52	7.46	.06	38.5	42.5	4.0	31.6	30.2	1.4
10.	7.43	7.39	.06	42.5	48.5	6.0	28.2	29.5	1.3
11.	7.41	7.35	.06	36.7	41.0	5.3	23.3	23.2	0.1
12.	7.40	7.39	.01	40.6	47.0	6.4	25.3	28.6	3.3
13.	7.43	7.39	.04	41.4	48.0	6.6	27.5	29.2	1.7
14.	7.41	7.37	.04	49.2	56.0	6.8	31.3	32.6	1.3
15.	7.44	7.42	.02	42.0	46.5	4.5	28.5	30.3	1.8
16.	7.42	7.39	.03	48.0	51.0	3.0	31.2	31.0	0.2
17.	7.49	7.44	.05	46.0	52.6	6.6	35.2	35.4	0.2
18.	7.40	7.40	.00	49.0	50.0	1.0	31.0	31.1	0.1
19.	7.38	7.35	.03	74.0	79.0	5.0	44.2	44.2	0.0
20.	7.41	7.36	.05	40.5	52.5	12.0	25.8	30.0	4.2
21.	7.43	7.38	.05	47.5	57.5	10.0	29.2	34.3	5.1
22.	7.38	7.34	.04	41.5	50.0	8.5	24.7	27.1	2.4
23.	7.43	7.39	.04	46.5	56.0	9.5	28.6	34.1	5.5
24.	7.41	7.35	.06	37.4	46.4	9.0	23.8	25.7	1.9
25.	7.49	7.45	.04	30.5	39.0	8.5	23.4	27.1	3.7
26.	7.42	7.37	.05	47.0	57.0	10.0	30.6	33.1	2.5
27.	7.40	7.37	.03	59.0	70.0	11.0	36.8	40.8	4.0
28.	7.48	7.36	.12	35.5	48.6	13.1	26.2	27.8	1.6
29.	7.37	7.32	.05	60.0	74.0	14.0	35.1	38.3	3.2
30.	7.42	7.36	.06	36.5	54.0	17.5	23.8	31.9	8.1
31.	7.53	7.40	.13	36.0	52.0	16.0	30.2	32.5	2.3
32.	7.40	7.33	.07	43.5	62.0	19.0	27.4	32.8	5.4
33.	7.45	7.38	.07	33.5	47.0	13.5	23.4	28.1	4.7
34.	7.46	7.42	.04	36.3	48.4	12.1	25.9	31.5	5.6
35.	7.43	7.29	.14	36.8	60.0	23.2	34.2	29.3	4.9
36.	7.53	7.45	.08	34.0	51.0	17.0	28.6	35.6	7.0
37.	7.39	7.34	.05	43.7	61.0	17.3	26.6	34.5	7.9
38.	7.17	7.14	.03	77.8	102.0	24.2	30.0	36.0	2.7
39.	7.37	7.33	.05	42.0	57.0	15.0	27.8	32.0	4.2
40.	7.25	7.17	.08	51.5	74.0	22.5	23.5	28.0	4.5
41.	7.51	7.45	.06	48.8	60.0	11.2	39.8	44.0	4.2
42.	7.40	7.34	.06	39.4	57.0	17.6	25.4	32.0	7.5
43.	7.43	7.37	.06	36.6	59.0	22.4	25.4	35.0	9.6
44.	7.49	7.40	.09	36.4	58.0	21.6	28.8	36.0	7.2
45.	7.43	7.38	.05	50.5	64.0	13.5	34.5	39.0	3.5
46.	7.41	7.34	.07	37.1	53.9	16.8	24.0	30.6	6.6
47.	7.38	7.25	.13	23.3	35.7	12.4	14.2	16.0	1.8
48.	7.29	7.29	.00	87.0	98.0	11.0	41.3	48.0	6.7
49.	7.34	7.31	.03	29.1	41.1	12.0	16.2	22.0	5.8
50.	7.37	7.24	.13	28.5	51.4	22.9	17.0	23.3	6.0

On the basis of the small amount of data presented here, clinical experience, and information derived from the literature, I would generally favor arterial blood as a reference point for all acid-base disturbances, even those of metabolic origin. This is not to exclude the use of serial venous pH and PCO_2 measurements made with the reservations mentioned above. I believe that the door should be kept open on this point, and other experienced opinions obtained. Again, this may be a matter of judgement--reinforcing my original concept of the field of pH and blood gas measurement.

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BLOOD SAMPLING AND HANDLING IN THE DETERMINATION OF BLOOD pH AND BLOOD GASES

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The collection and handling of blood for the determination of blood pH and blood gases require that the source (arterial, capillary or venous), sampling site, collection materials and storage conditions be precisely and clearly defined in order to ensure that a proper sample is presented to the analytical unit.

Although the source of the blood must sometimes be selected on a purely pragmatic basis, arterial blood is the preferred type of sample and will reflect the true status of the pulmonary system with respect to pH and blood gas content. Collection of the sample, usually from the brachial, femoral or radial arteries, requires a high degree of expertise. The patient must be relaxed and the blood drawn with a minimum of trauma. Multiple arterial samples are not recommended, but, when several samples might be required, a Cournand needle or Seddinger catheter can be employed.

Blood collected *via* a vasodilated capillary bed has been reported to be virtually equivalent to arterial blood [1-3][1]. The proper technique for collection of a capillary sample (usually from the earlobe, finger or heel) is to warm the site thoroughly by immersion in warm water, then allow the blood to flow into a capillary tube without impairing or forcing the blood flow. The results on arterialized capillary blood have been reported to show good agreement with samples taken from a true arterial source, although a notable exception has been reported: Po_2 arterial versus capillary measurements were reported to be in poor agreement [5].

Venous blood can be employed for the routine measurement of CO_2 content. However, it is well documented that oxygen tension can be considerably different than in arterial blood [10-12,14]. Some reports have stated that reasonably satisfactory samples can be obtained by the application of heat to the source to create "arterialization." One monograph recommends that blood be taken from a vein on the back of a hand that has been subjected to 46-47 °C heat for 10-15 minutes [11]. The use of a light tourniquet is acceptable, but it should be released during the drawing period. It has been reported that, when these conditions are closely followed, good approximation of arterial blood is achieved for pH and Pco_2, although Po_2 results can be significantly different.

The collection device most frequently recommended is the glass-type syringe with matching barrel and plunger. A light coating of heparin anticoagulant solution should be used for lubrication as well as for preventing clot formation. Samples are always drawn under anaerobic conditions. Plastic syringes cannot be used as the porosity of the plastic creates a potential for CO_2 and O_2 to diffuse into the plastic. Vacutainers containing sodium heparinate have been shown to be acceptable [8]. Other anticoagulants are unacceptable, and oil or lubricants must never be used.

It is recommended that the blood sample be placed in ice water if analysis cannot be performed within 20 minutes [2]. It is reported that blood pH will not change more than 0.01 pH units when kept at 4 °C for up to four hours. Pco_2 is a more labile determination,

[1]Figures in brackets indicate the literature references at the end of this paper.

for which it is recommended that the sample stored on ice be analyzed within 30 minutes. The sample must be kept from exposure to room air by inserting the collection needle into a cork or rubber stopper or by using any appropriate covering device. The sample must be remixed just prior to proceeding with the determinations. Mixing is best accomplished by adding a few drops of mercury to the sample, both to assist in completely mixing the anticoagulant and remix to the sample at a later time.

Numerous studies have compared the results of tests in which arterial blood and venous and capillary bloods, arterialized by a variety of techniques, were used. Paine, *et al.* [4] investigated a group of 29 hospitalized patients by making a comparison between arterial samples, venous samples and arterialized venous samples. The arterialized venous samples were obtained by prewarming a vein on the dorsum of the hand for 15-20 minutes and were collected with a tourniquet applied to the wrist. The pH values were on the average 0.022 units lower than arterial blood, while the Pco_2 was 2.86 mm Hg lower. It was concluded that this arterialized sample is satisfactory for the estimation of arterial pH.

In a separate study, Jung, *et al.* [14] made comparisons of arterial and venous bloods collected simultaneously with both syringes and vacutainers. Capillary blood was also used in some of these studies. The pH, Pco_2, bicarbonate, total buffer base and base excess were all satisfactory on capillary blood, but Po_2 was not acceptable. The study stated that blood obtained by Vacutainer was not adequate for any of the determinations, but haparinized venous blood could be substituted for arterial blood except for the Po_2 determination.

A study by Spock, *et al.* [13] showed that Po_2 could be accurately determined on capillary samples if a proper technique was employed. An excellent correlation with arterial samples was shown in this study. These samples were obtained by thumb prick after the thumb had been warmed at 40 °C. The samples were analyzed promptly and were representative of a number of disease states.

In yet another study, Koch [11] has shown that capillary blood from the middle finger, when arterialized by warming at 45 °C, was comparable to arterial blood for measuring pH, Pco_2 and bicarbonate concentration.

One of the favored collection sites for blood pH and blood gas determinations is from the earlobe. Laughlin, *et al.* [6] have shown that by warming the earlobe with an electric heater, Po_2 values could be obtained quite comparable with Po_2 values in arterial blood. In the same study samples from finger punctures gave less satisfactory Po_2 values than those from earlobe punctures.

Langlands, *et al.* [7] compared Po_2, Pco_2 and pH values brachial artery and earlobe blood. Their results showed that values in arterialized earlobe blood corresponded very closely to arterial blood.

Gambino [8] has utilized the earlobe as a site for collection of capillary blood. He described a rubber-cup collection system and compared his results with arterial values. No significant differences were observed between the capillary blood and arterial blood values for pH, CO_2 content, Pco_2 and oxygen saturation.

Siggaard-Anderson, *et al.* [9] have done studies on capillary blood and state that pH shows a standard deviation of 0.006 pH units and Pco_2 accuracy of ± 2 percent.

In summary, it would appear that although the ideal situation is to obtain arterial blood, venous or capillary blood can serve as a valid substitute providing the collection site is carefully chosen and prewarmed and the specimen is carefully collected and properly cared for prior to analysis.

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NON-ANALYTICAL SOURCES OF LABORATORY ERROR IN pH AND BLOOD GAS ANALYSIS

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Laboratory error includes more than just the analytical error associated with instrumental measurement. Properly, it should be defined as any error from the ordering of a test procedure until the interpretation of results. In our experience, the non-analytical sources of error are more severe than the instrumental and harder to document and eliminate [1][1]. The purpose of this presentation is to review some of these non-analytical sources of laboratory error in pH/blood gas measurements in an effort to alert both the laboratorian and clinician to these problems.

1. Choice of Collection Site

A. Arterial *versus* venous blood

The differences between arterial and venous blood found by various studies are shown in table 1. Comparisons of arterial and capillary blood obtained without any attempt to arterialize the capillary collection site are also shown. It is evident that venous blood obtained from an arm vein has a lower pH than arterial blood. The magnitude of this difference varied from study to study but appears to be ∿0.05 pH unit. When capillary blood is compared to arterial blood, discrepancies between the conclusions of the studies are found. This is probably due to differences between the patients studied as will be discussed in the next section. Only small differences between arterial and capillary blood were found in studies of patients under general anesthesia [6,10,11], and were attributed to vasodilation and high cutaneous blood flow caused by anesthesia. These phenomena have been reported to help to arterialize the capillary blood [8]. In newborns, very poor correlations were found between arterial blood and blood obtained from an unwarmed heel [15].

The Pco_2 of venous blood is higher than arterial blood. The exact magnitude of the mean difference varied from study to study (table 1) but appears to be between 5 and 10 mm Hg. Again capillary blood agreed more closely with arterial blood than did blood from an arm vein. An important exception to this finding was the poor correlation of capillary (unwarmed heel) and arterial blood for Pco_2 in newborns [15]. Very large differences in Po_2 and O_2 saturation between arterial and venous blood have been observed [4,7] with smaller differences between arterial and capillary blood [12,14].

The data indicates that for most purposes, substitution of venous blood for arterial blood will not cause clinically significant differences for pH, may cause clinically significant differences for Pco_2, and will invalidate measurements of oxygen status.

B. Arterial *versus* arterialized blood

Because of the possible risk and discomfort of arterial puncture [16,17], many attempts to arterialize venous blood by means of warming or vasodilation have been

[1]Figures in brackets indicate the literature references at the end of this paper.

Table 1. Differences between arterial and venous blood.[a, b]

Year	Venous site	pH	Pco_2 (mmHg)	Po_2 (mmHg)	O_2 Saturation (%)	Number and type of subjects	Reference
1963	Vena Cava	-.005	+2.4			4 patients with suspected cardiac defects	2
1974	Central Venous	+.041				501 critically or seriously ill patients on 100 percent O_2	3
	Central Venous		-7.5			328 critically or seriously ill patients on 100 percent O_2	3
1942	Int. Jug.	+.053	-10		31.7	50 normals	4
1959	Arm	+.013				30 resting patients	5
1964	Antecubital	+.06	+7.4			30 patients under anesthesia	6
1966	Cephalic	+.033	-4.7	+27.3		42-43 patients	7
1959	Hand or Wrist	+.051	-8.3			14 patients	8
	Hand or Wrist	+.01	-1.2			7 patients with warm extremities	8
	Hand or Wrist	+.002	-1.1			10 patients under anesthesia	8
1961	Hand or Wrist	+.044	-3.4			14 patients	9
	Hand or Wrist		-1.6			10 patients under anesthesia (13 samples)	10
1962	Capillary, earlobe	-.001	+0.4			31 patients under anesthesia	11
1964	Capillary, earlobe	+.01	-1.0			15 patients under anesthesia	6
	Capillary, earlobe			+9		8 patients	12
1962	Capillary, finger	-.004	+0.2			20 patients under anesthesia	11
1963	Capillary, finger	-.04	+6			4 patients with suspected cardiac defects	2
1964	Capillary, finger	+.03	-7.3			15 patients under anesthesia	6
1965	Capillary, finger	+.009	-2.8			13 patients	13
1968	Capillary, thumb			+8.5		14 patients	14
1964	Capillary, heel	.15(max) very poor correlation	-48(max) very poor correlation			23 newborns	15

[a] All differences are expressed as arterial-venous

[b] All capillary blood was non-arterialized

attempted. These results are summarized in table 2. Direct comparisons of arterial, arterialized earlobe, and arterialized thumb pulp have been performed for Po_2 measurements. Good agreement between arterial blood and blood prepared by massaging the earlobe with nicotinic acid (Trafuril^R) was obtained. Blood obtained from the earlobe prepared by massage without nicotinic acid, the untreated thumb pulp, or the heated hand was found to differ significantly from arterial blood [14]. Other direct comparisons also found arterialized blood from the earlobe to be superior to that from the finger [12,27].

Table 2. Differences between arterial and "arterialized" blood.[a]

Year	Sample site	pH	Pco_2 (mmHg)	Po_2 (mmHg)	O_2 Saturation (%)	Number and type of subjects	Reference
1959	Dorsal hand vein	+.008	-1.9			14 patients	8
1961	Dorsal hand vein	+.018	-0.1			14 patients	9
1972	Dorsal hand vein	+.005	-1.1	16 (Po_2>70) 5.9 (Po_2<70)		8 patients and 5 normals	18
1944	Earlobe			0.5		11 patients	19
1961	Earlobe	-.002				22 patients	20
	Earlobe		+0.5			10 patients under anesthesia (21 samples)	10
1964	Earlobe			3		33 patients	12
	Earlobe				Good agreement	19 patients	21
	Earlobe				0.1	20 patients	22
1965	Earlobe				0.3	21 patients	23
	Earlobe	-.006	+1.0	0.6		14 patients and 2 normals	24
1966	Earlobe			r = 0.93		16 patients	25
1967	Earlobe	-.001	+0.3			17 patients (respiratory insufficiency)	26
1968	Earlobe			0.2 (Po_2, 34-103)		42 patients	27
	Earlobe			90 (Po_2, 222-603)		12 patients	27
	Earlobe			0.7 (Po_2, 40-91)		22 patients during exercise	27
1968	Earlobe			0.5		14 patients	14
1972	Earlobe	-.002	-0.8	0.4		25 normals	28
1973	Earlobe	+.006	-1.4	0.2		84 patients, preoperative, operative, and postoperative	29
1961	Ear or finger	+.003	0.2		0.1	13 patients	30
	Ear or finger	+.003				8 patients after one minute exercise	30

Table 2. Differences between arterial and "arterialized" blood (continued).

Year	Sample site	pH	Pco_2 (mmHg)	Po_2 (mmHg)	O_2 Saturation (%)	Number and type of subjects	Reference
	Ear or finger		-0.2			5 patients after one minute exercise	30
1961	Finger			6.4		43 patients	26
1963	Finger	-.011	+2.2			47 patients	2
1965	Finger	+.034	-0.3			30 patients	13
1966	Finger	-.004		0.2	0	20 patients	31
1962	Finger and heel		1.0	0.2		22 patients	32
1964	Heel	+.027	-5.9			20 newborns 0-1 hour	15
1964	Heel	+.01	-1.8			20 newborns 1-3 hours	15
	Heel	+.006	-0.7			34 newborns over 3 hours	15
1967	Heel	+.026	-6.2			34 newborns 30 minutes to 24 hours	33
	Heel	+.002	-0.8			42 newborns 2-6 days	33
	Heel			9.5		5 hours to 6 days	33
1969	Heel	+.007	-8.3	6.3		45 newborns 3-95 hours	34
1973	Heel	r = 0.99	-(10-15)	r = 0.86		37 newborns older than 3.5 days	35
1965	Scalp	Small	Small	Small		48 patients	36

[a]All differences expressed as arterial-"arterialized".

As table 2 indicates, in almost all studies of adults the capillary method gave results equivalent to arterial blood. In newborns, however, differences have been found and it appears that this technique is not valid in very young children (less than a day) and questionable even in somewhat older children. The lack of agreement between arterial and arterialized capillary blood in newborns has generally been ascribed to poor circulation and warnings about using arterialized capillary blood in any patient with circulatory shock have been presented [12,25,27,37]. Observations by Banister [34], however, have suggested the presence of edema of the foot, rather than circulatory status, as the important factor in obtaining valid results from capillary blood in children.

Prior publications [38,39] have presented conclusions which are in keeping with the data reviewed here. These conclusions were that arterialized capillary blood is equivalent to arterial blood except for the very young, newborns with respiratory distress syndrome, and patients in shock. Unfortunately, these categories represent a number of the patients in whom a valid capillary sample would be highly desirable.

2. Collection Containers

Three types of collection containers for macro pH/blood gas determination have been suggested; glass syringe, plastic syringe, or vacuum tube. In an early study, no dif-

ference between the pH of whole blood obtained from a vacuum tube and that sampled directly from the patient was noted. In the same study, no differences in plasma CO_2 content between sedimented blood from a syringe and centrifuged vacuum tubes was observed. The O_2 saturation of arterial blood collected in a syringe or in a vacuum tube was also found to be the same [40]. Fleisher and Schwartz [41] noted a poor correlation between the usual vacuum tubes and glass syringes for arterial blood gas determinations and designed a nitrogen filled vacuum tube. Comparison of this tube and glass syringes, showed excellent agreement for Pco_2 and Po_2, and only a +0.01 difference for pH [41]. These nitrogen filled tubes were also tested by Lang, *et al.* [42], who found them to be severely contaminated with oxygen. For blood samples, good correlations for pH and Pco_2 were observed, but significantly higher Po_2 values were found with the vacuum tube. Further work by these authors showed the unsuitability of the presently available vacuum tubes for measurement of Po_2 and oxygen saturation [43].

A. Glass *versus* plastic

Saline equilibrated to a high Po_2 (~700 mm Hg) showed no decline in measured Po_2 when kept in glass syringes, but showed a decline of 21.6 percent after 3 hours and 67 percent after 48 hours when stored in plastic syringes. Blood tonometered to high Po_2 values (~600 mm Hg) also showed a greater decay in plastic syringes as compared to glass. The differences in decay rate were apparent in fifteen minutes [14]. The diffusion of oxygen and carbon dioxide from plastic syringes has been studied. The percent change in gas content per hour varied considerably (over 100 percent) between the two types of plastic syringes tested. For the better syringe type, the changes at room temperature (in percent change of gas content/h) were 0.034 and 0.082 for O_2 and CO_2, respectively. When the syringes were kept on ice these changes were 0.128 and 0.154. These authors expected that the changes would be considerably slower when the syringe content was liquid and did not feel that the decay would be clinically significant [44]. Measuring the Po_2 of deoxygenated water gave higher results for samples in plastic syringes than for glass (37 mm Hg compared to 13 mm Hg) [42]. Laver and Seifen [45] have noted that plastic syringes maintain a high Po_2 as well as glass syringes for up to 2 hours, but recommend glass syringes if delay in analysis is expected.

Scott, *et al.* [46], found that when water tonometered to 96 percent O_2 was sampled in 6 different types of plastic syringes, large decreases were found when the results were compared to the same sample in glass syringes. Similarly, large increases were found when sampling water at 0 percent O_2. Syringes made of polystyrene showed considerably greater changes than those made of polypropylene or S.A.N. co-polymer. Tonometered blood samples showed the following changes (in mm Hg) after 11.5 minutes, -68, 0.4, and 0.0 at an initial Po_2 of 680, 102, and 68, respectively. These authors presented evidence that the loss or gain in O_2 was due to diffusion into the walls of the syringe rather than diffusion through the syringe. These authors estimated the magnitude of errors expected under various conditions. This data is presented in table 3, and extimates the influence of initial Po_2, temperature of storage, pH, hemoglobin and barometric pressure on the error introduced by the use of plastic syringes. It is evident that the error introduced will depend on a number of factors and the acceptibility of plastic syringes will depend on the application. The above study also noted that interchangeable glass syringes were not as effective as glass syringes with matched barrel and plunger.

The above data indicates that under most conditions, the interchange of glass or plastic syringes will not affect the clinical utility of the oxygen measurement [44-46]. The excellent work of Scott (table 3), however, does show that in some circumstances inaccurate results will be obtained when plastic syringes are utilized. An example of this situation may be the severely anemic patient with alkalosis who is on a respirator. There are advantages to the laboratory in using plastic syringes such as cost, safety, and seal reliability. When mixing the blood, the plunger of more than one glass syringe has fallen out if care is not taken. The area of collection containers appears to be in need of some fresh approaches which would retain the accuracy of glass syringes but with the convenience of plastic ones. Regardless of whether glass or plastic syringes are used, the addition of mercury to facilitate mixing the blood is not recommended due to the danger of peripheral embolism [47].

Table 3. Computed oxygen exchange of blood stored in plastic syringes [46].

	Storage time (min)	Initial tension (mmHg)		Change of tension (mmHg)	Change of tension (% initial)	Change of content (% initial)
"Normal" conditions	30-60	650		-106.3	-16.4	-1.4
		300		-21.3	-7.1	-0.3
		100		5.8	5.8	0.4
		33		0.3	0.9	0.9
Effect of storage time	2	650		-30.3	-4.7	-0.4
			Ta			
Effect of ambient temperature during storage (Ta)	30-60	650	37	-129.1	-19.9	-1.7
		650	4	-83.3	-12.8	-1.1
		100	37	3.3	3.3	0.2
		100	4	7.6	7.6	0.5
			Hb			
Effect of Hb	30-60	650	15	-106.3	-16.4	-1.4
		650	5	-107.5	-16.5	-3.6
		100	15	5.8	5.8	0.4
		100	5	12.7	12.7	1.1
			pH			
Effect of pH	30-60	300	7.7	-24.3	-8.1	-0.4
			6.8	-10.8	-3.6	-0.2
			Pb			
Effect of barometric pressure (Pb)	30-60	100	780	6.1	6.1	0.4
			710	5.2	5.2	0.3
			600	3.7	3.7	0.2

Notes: Syringes: 5 ml or 2 ml, initially in equilibrium with ambient air.
Exchange factor: 8×10^{-4} (ml/100 m)/mmHg for 30-60 minutes storage and 2×10^{-4} for 2 minutes.
Normal conditions: Hb = 15 g/100 m, pH = 7.4, Pb = 760 mmHg, initial blood temperature = 37 °C, ambient temperature = 22.5 °C.

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3. Effect of Anticoagulants

While serum and plasma give comparable results for pH [40] and CO_2 content [48], whole blood is necessary for oxygen analysis. The use of citrate [40,49], oxalate (NH_4^+ and K^+) [40], or EDTA [7,40] as anticoagulants has been found to cause low results for pH. The use of potassium oxalate alone as anticoagulant caused an increase in pH [40,50]. This increase in pH was shown to be related to chelation of calcium [50]. Heparin has almost no effect on pH (1 mg/ml ≈ -0.003). This small effect was the same in lipemic blood but no data concerning the storage of such specimens was presented [51]. In an effort to inhibit glycolysis, heparin-fluoride mixtures have been utilized. In one study, no effect of fluoride on oxygen consumption was found [52] while in others a decrease in decay of Po_2 of 1/4 to 1/3 was observed [53,54]. Paradoxically, fluoride completely eliminated the rise in Pco_2 with storage even though the Po_2 was only partially affected [53]. No differences in the pH of four blood specimens were found when heparin-fluoride was utilized [49]. The addition of fluoride in amounts sufficient to inhibit glycolysis lowers the pK' of blood by 0.01 units due to a salt effect [55]. Fluoride (5 mg/ml) caused an increase in pH of 0.03 and a fall in Pco_2 of 6 mm Hg. This effect was found to be concentration dependent [51]. Upon storage, blood containing fluoride reacted differently than blood without fluoride. When stored at 38 °C, the change in the pH of blood reached a minimum at 1/2-1 hour (0.01-0.02) and a maximum at 3-4 hours (0.17-0.19). This phenomena was concentration dependent, delayed at 22 °C, and was not due to leukocytes [51]. The addition of fluoride causes a rise in potassium which invalidates its use for potassium measurement [56]. In our laboratory, potassium analysis is being requested on the same sample of blood as pH/blood gas with greater frequency. The rise in potassium and the above data on changes in pH during storage suggest that fluoride not be used as an additive to samples for pH/blood gas measurement. It is therefore recommended that heparin be used as the sole anticoagulant for pH/blood gas analysis.

4. Stability *In Vitro*

In this section the stability of pH, Pco_2, CO_2 content, and Po_2 in blood under varying conditions is reviewed. Since anticoagulants other than heparin can have a deleterious effect on the measured values of these quantities, only data obtained with this anticoagulant will be considered. As will be discussed later, the temperature of measurement can affect results and therefore some early data where the temperature control is suspect [57] will not be considered.

A. pH

The average decay rates for pH (-pH/h) found in various studies are shown in table 4. This decay rate has been shown to be independent of hemoglobin concentration but very dependent on the number of leukocytes [51]. It is evident that the decay in pH is quite temperature dependent and this dependence has been shown to follow the Arrhenius relationship [62]. Exposure to air by dropping 50 µl of blood onto a glass slide resulted in a rise of only 0.01 pH after 2 minutes [51]. The small decay rate for pH should not cause problems in routine handling of blood specimens if they are placed in ice water after being obtained. Such treatment has been stated to result in decay rates of no more than -0.008 pH/h even in patients with leukemia [63].

B. Pco_2

As shown in table 4, Pco_2 in blood has been found to increase during storage. For blood stored at 4 °C the average increase is less than 0.7 mm Hg/h. The magnitude of this increase has been found to be related to the number of white blood cells present but not to the number of reticulocytes [53]. This small increase will not usually be clinically

Table 4. Stability of pH, Pco_2, CO_2 content and Po_2.

Parameter	Year	Storage temperature	Rate of change	Number of experiments	Comments	Reference
pH	1956	?	0.060 (-pH/h)	?	Data not given	55
	1961	38 °C	0.062 (-pH/h)	10	Linear for 3 hrs and dependent on leukocytes but not hemoglobin	51
		22-24 °C	0.024 (-pH/h)	5	Linear for 3 hrs and dependent on leukocytes but not hemoglobin	
		0-4 °C	0.006 (-pH/h)	4	Linear for 3 hrs and dependent on leukocytes but not hemoglobin	
	1965	2-4 °C	0.006 (-pH/h)	17	Based on 4 hours	13
Pco_2	1961	38 °C	4.8 (mmHg/h)	10	Linear for 3 hours	51
		22-24 °C	2.5 (mmHg/h)	5		
		0-4 °C	0.6 (mmHg/h)	4		
	1965	37 °C	6.6 (mmHg/h)	?		52
		4 °C	0.7 (mmHg/h)	?		
		2-4 °C	0.2 (mmHg/h)	5		13

Table 4. Stability of pH, Pco_2, CO_2 content and Po_2 (continued).

Parameter	Year	Storage temperature	Rate of change	Number of experiments	Comments	Reference
CO_2 content	1959	room?	0	?	No effect after 2 hours	40
	1971	room?	0	1	No effect after 2 hours	58
Po_2	1961	37 °C	175 (-mmHg/h)	5	Initial Po_2, over 400	52
			20 (-mmHg/h)	5	Initial Po_2, ∿ 100	
	1965	37 °C	3.4 (-mmHg/h)	?	Initial Po_2, 51-93	59
		22-24 °C	1.8 (-mmHg/h)	?	Rate was not constant, and was calculated from 1 hr data	
		1 °C	1.7 (-mmHg/h)	?	Rate was not constant, and was calculated from 1 hr data	
		37 °C	121 (-mmHg/h)	?	Initial Po_2, 360-670, rate was not constant and was calculated from 1 hr data	
		22-24 °C	16 (-mmHg/h)	?	Initial Po_2, 360-670, rate was not constant and was calculated from 1 hr data	
		1 °C	1.7 (-mmHg/h)	?	Initial Po_2, 360-670, rate was not constant and was calculated from 1 hr data	
		37 °C	156 (-mmHg/h)	55	High initial Po_2	53
		4 °C	30 (-mmHg/h)	10	High initial Po_2	
		37 °C	24 (-mmHg/h)	5	Initial Po_2, 60-100	53
		37 °C	48 (-mmHg/h)	16	Initial Po_2, 125-150	
		37 °C	162 (-mmHg/h)	55	Initial Po_2, 440-610	
	1966	4 °C	10 (-mmHg/h)	?		31
		room	450 (-mmHg/h)	89 samples 5 normals	Breathing 100% O_2. Rate is projected based on first 10 minutes	60
	1968	37 °C	180 (-mmHg/h)	?	Initial Po_2, 680	14
		24 °C	120 (-mmHg/h)	?	Initial Po_2, 680	
		4 °C	30 (-mmHg/h)	?	Initial Po_2, 680	
		37 °C	50 (-mmHg/h)	?	Initial Po_2, 143	
		24 °C	20 (-mmHg/h)	?	Initial Po_2, 143	
		4 °C	10 (-mmHg/h)	?	Initial Po_2, 143	
		?	180 (-mmHg/h)	?	Higher if leukocytosis present	61
	1968	0-4 °C	-0.5 (-mmHg/h)	10	Po_2, 52-92	27

significant but extensive information on patients with high levels of white blood cells, *e.g.*, leukemia, is not available.

C. CO_2 content

As expected from the data concerning pH and Pco_2, little or no change in blood CO_2 content has been found when the blood is kept unopened to air (table 4). The temperature

of centrifugation has been suggested as a possible small cause of error in CO_2 content measurements because of the different temperature coefficients of plasma and whole blood [64]. In two studies, however, no effect of the temperature of centrifugation (5 °C or 37.5 °C) was found [40,55]. Since the temperature of a centrifuge operated in an ambient temperature room is over 30 °C, this potential error source can be ignored.

A more significant stability problem occurs when plasma or serum is placed in the plastic sampling cups which are so commonly used. The loss of CO_2 in sample cups open to the air has been shown to average 3.5 meq/l for 15 samples after 1 hour. In one patient with a Pco_2 of 75 mm Hg, a 7 meq/l loss after 3 hours occurred [65]. Four techniques to minimize CO_2 loss to the atmosphere have been described. The oldest of these techniques is the use of oil to eliminate contact of the sample with the atmosphere. This technique has been criticized for two reasons: (1) since blood collected with and without oil gives the same results [48], its use during collection is superfluous; and (2) CO_2 is quite soluble in oil (see annotated bibliography in reference 66) and significant absorption of CO_2 occurs if the sample is mixed with the oil. Such mixing can occur during collection and/or centrifugation [67]. While covering the sample cups with oil does retard CO_2 loss to the atmosphere [68], the potential CO_2 loss to the oil makes its use unadvisable.

The second method consists of placing a plastic disk in the sample cup so that the sample is isolated from air. The sample probe then offsets the disk at the time of analysis. This system has resulted in no loss of CO_2 in 3 hours [69]. The third method is the use of added alkali (1 drop of 1 $\underline{N}$ NH_4OH) to decrease the Pco_2. This technique was found to stabilize the CO_2 content for up to 4 hours and was felt to be superior to the plastic disk method [65]. The fourth technique is the use of a metal or plastic plate to cover all the cups in a sample tray. Such equipment is commercially available but the author is unaware of any published studies on its effectiveness in retarding CO_2 loss.

The loss of CO_2 to the atmosphere during handling is certainly capable of affecting the clinical interpretation of CO_2 content measurements. Of the methods noted above for minimizing this phenomena, the alkali method of Gambino [65] appears to be best but further validation by other workers would be helpful.

D. Po_2

The stability of Po_2 in shed blood under various conditions is shown in table 4. It is evident that the stability of Po_2 is quite temperature dependent [14,25,53,54,59,70,71] and rapid chilling of all blood samples is strongly suggested. It is also evident that the rate of decay of Po_2 is highly dependent on the initial Po_2 [14,25,52, 53,59,71,72]. This has been attributed to the shape of the oxygen dissociation curve [53]. The O_2 decay rate in shed blood is not affected by total hemolysis of the blood sample [53].

The source of the oxygen consumption in blood specimens has been the subject of study. In one study the oxygen consumption of normal mature erythrocytes was found to be negligible but blood with abnormal numbers of reticulocytes had an oxygen consumption proportional to the percentage of reticulocytes present [73]. This increase in oxygen consumption with increase in percentage of reticulocytes has been confirmed by others [53,74]. The rate of oxygen consumption has also been related to the white cells. Figure 1 shows the data of Lenfant and Aucutt [53] relating oxygen consumption to the white blood count. Normal and abnormal white cells are included in this data but no comparison of their oxygen consumption was made. Higher oxygen consumption in blood from leukemic subjects has been noted by another worker as well [61]. Because of the dependency of O_2 consumption on the cellular components of the blood, correction factors for storage time are not recommended and prompt analysis of chilled specimens appear the best way of handling blood for accurate Po_2 measurements.

Exposure of blood to air for short periods (10-30 seconds) results in significant changes in Po_2 toward the Po_2 of room air [25]. The presence of air bubbles (up to 10 percent of the total syringe volume) has little effect on Po_2 until the surface area is increased by mixing [71]. The presence of air bubbles in blood samples has been blamed for discrepancies in interlaboratory surveys for Po_2 [75]. Since whole blood is mixed prior to pH/blood gas analysis to avoid suspension of the cells, great care should be exerted to exclude air bubbles at the time the sample is obtained.

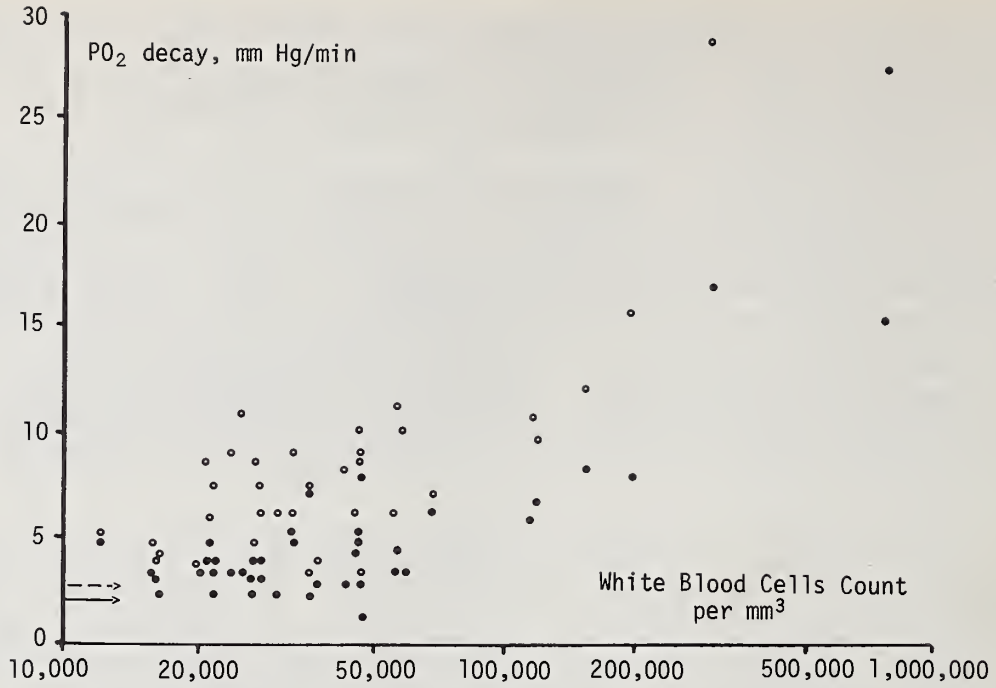

Figure 1. Po_2 decay as a function of the white cell count. Black circles are for samples containing NaF. Arrows indicate the Po_2 decay of samples with normal white cell counts without (- - →) and with (⟶) NaF. (From Lenfant and Aucutt [53] with permission of *J. Appl. Physiol.*)

5. Effect of Measurement Temperature

pH, Po_2, and Pco_2 values are all dependent on the measurement temperature. Since virtually all commercial pH/blood gas equipment measures these quantities at 37 °C, an error is introduced when the temperature of the patient at the time the sample is obtained deviates from this value. The work of various authors has been reviewed and a simplified table derived for temperature correction of pH, Po_2, and Pco_2 [76]. This table is shown as table 5 and is recommended as a guide to temperature effects on pH and blood gas measurements.

6. Effects of Red Cells on pH Measurement

No effects of hematocrit on the measurement of Po_2 or Pco_2 have been reported. However, differences in measured pH between plasma and whole blood have been observed (plasma-whole blood ≈0.01) [55,77]. The relationship between pH and hematocrit (0-100) in 5 blood samples has been elucidated and the cause of the small pH discrepancies was felt to be due to differences in residual liquid junction potentials between plasma and whole blood [77]. The magnitude of this error is quite small and except in highly unusual circumstances will not be clinically significant.

7. Effects of Dilution

A 12-13 percent dilution of blood with physiological saline produced no significant change in pH but resulted in a fall in Pco_2 of 16 percent and a fall in plasma bicarbonate of 15 percent [51]. Another study showed an increase in pH of 0.023 pH unit after a 20 percent dilution with physiological saline. The addition of calcium (5 meq/l) to the saline eliminated this dilution error [78]. A 33 percent dilution of blood with isotonic saline caused no change in pH, a decrease of 3.8 mm Hg (4 percent) in Po_2 and a decrease of 9.2 mm Hg (24 percent) in Pco_2 [24]. The above data indicate that pH and Po_2 exhibit little

Table 5. Temperature-correction factors for blood pH and gas measurements [76].[a]

Patient's temperature		pH	P_{CO_2} (%)	P_{O_2} (%)
°F	°C	(Add to observed values)		
110	43	-.09	+22	+33
109	42.5	-.08	+21	+32
108	42	-.07	+19	+30
107	41.5	-.07	+17	+27
106	41	-.06	+16	+25
105	40.5	-.05	+14	+22
104	40	-.04	+12	+19
103	39.5	-.04	+10	+16
102	39	-.03	+8	+13
101	38.5	-.02	+6	+10
100	38	-.01	+4	+7
98-99	37	None	None	None
97	36	+.01	-4	-7
96	35.5	+.02	-6	-10
95	35	+.03	-8	-13
94	34.5	+.04	-10	-16
93	34	+.04	-12	-19
91	33	+.06	-16	-25
90	32	+.07	-19	-30
88	31	+.09	-22	-35
86	30	+.10	-26	-39
84	29	+.12	-29	-43
82	28	+.13	-32	-47
81	27	+.15	-34	-51
79	26	+.16	-37	-54
77	25	+.18	-40	-57
75	24	+.19	-43	-60
73	23	+.21	-45	-63
72	22	+.22	-48	-65
70	21	+.24	-50	-67
68	20	+.25	-53	-70

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change, even with high dilution, but P_{CO_2} and CO_2 content change in proportion to the degree of dilution. Unless a large volume of anticoagulant is used, this should not introduce a significant error. In sampling from indwelling catheters, care should be taken to remove the fluid filling the catheter before obtaining the blood specimen if accurate assessment of CO_2 status is desired.

8. Effect of Stasis

No difference in pH between venous blood obtained without a tourniquet and blood obtained with the tourniquet in place for 2 minutes was found in 5 subjects. It was emphasized that the tourniquet should be left in place since the stasis produced is at the capillary level and is not reflected in the vein until the tourniquet is released [79]. These results have been confirmed and it was noted that the tourniquet can be left in place for 5 minutes without exercise (hand pumping) but that after tourniquet release the pH decreased precipitously [5].

9. Miscellaneous Effects

Arterial pH was found to decrease by over 0.2 pH units after exhausive exercise and took over 30 minutes to recover. Pco_2 decreased by approximately 5 mm Hg as well [80]. Continuous monitoring of blood pH has shown small fluctuations with each group of respirations and has shown that hyperventilation results in a large increase in pH [81]. Warnings concerning increases in pH and decreases in Pco_2 during hyperventilation due to the pain or anxiety of arterial puncture have been presented [82]. Pco_2 values obtained in the sitting or standing position are 3-4 mm Hg lower than those obtained while recumbent [83]. The above data suggest that pH/blood gas studies should be performed on subjects known to be at rest unless special investigations are being performed. The injestion of food has been shown to cause an increase in arterial pH of 0.015 to 0.03 with no change in Pco_2. The Pco_2 was found to rise with sleep (5 mm Hg maximum) and the pH to decrease [84]. These changes associated with sleep and meals are small but should be thought of when it is decided when to obtain a sample for pH and blood gas analysis. Capnography (recording of expiratory CO_2-curve) did not alter the measured values for Po_2 or Pco_2 in patients with chronic lung diseases [85]. Halothane caused a slow response of some Po_2 electrodes and could cause erroneous Po_2 results with such electrodes [86]. Further work on this problem with more modern Po_2 electrodes has not yet been reported.

10. Conclusion

Advances in instrumentation have resulted in reliable, commercially available systems for the analysis of pH and blood gases. The future will probably see even more reliable instruments and these determinations will become more commonly performed than they are today. As the analytical component of laboratory error decreases it will be even more important for the clinician and laboratorian to appreciate the myriad of other factors which can lead to "laboratory error". In reviewing this information, it was obvious that for proper interpretation of laboratory data more knowledge needs to be obtained regarding these factors. Factors such as composition and design of collection devices, changes in values when storing blood with abnormal cells, and laboratory sample handling for CO_2 content require further careful investigation.

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INSTRUMENT SPECIFICATIONS

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Before I give you my specifications for laboratory instruments I want to show you what has happened to the number of blood gas assays performed at our hospital from 1970 through 1974 (table 1).

Table 1. Blood gas tallies.

	Central			Peripheral
	Day	Evening	Night	
1970	5,639	9,824		
1971	7,034	13,593		
1972	8,464	11,794	3,083	
1973	7,505	8,812	4,756	
1974	8,292	9,732	5,053	13,356

The data in table 1 show that in 1970, we performed a little over 15,000 blood gas analyses whereas in 1974 we performed 36,433 such assays. More important than the short doubling rate is the distribution of the assays. They do *not* concentrate on the day shift or on weekdays. Instead, the load is rather uniform throughout a 24-hour period, 7 days a week. In addition, table 1 shows preliminary data for assays performed outside of the main laboratory. In 1974, we had 2 peripheral blood gas machines located in intensive care units. Recorded assays performed on these 2 instruments by non-laboratory staff came to 13,356 in 1974. However, the actual number of such assays was probably more than 3 times that number since most emergency assays are not logged. In 1975, with the availability of instruments which incorporate automatic washout, Dr. Philip Altman and I have expanded peripheral coverage on the adult service to four locations utilizing Instrumentation Laboratory's 513 series.

Given the above data, and the obvious need for "bedside" measurements, the specifications for a practical clinical blood gas instrument become more obvious. Such an instrument should:

- Have automatic calibration.
- Have one simple sampling step.
- Be self cleaning.
- Require as small a sample size as possible, preferably less than 200 μl.
- Be as small as possible in physical size.
- Consume as little gas and reagent as possible, yet maintain constant calibration and be ready for instant use at any time.
- Be operable by non-laboratory personnel, including non-technical personnel such as clerks and nurses' aides.

I have outlined the functional characteristics of my ideal instrument. What about the required accuracy? What are the maximum error limits tolerable in a peripheral blood gas unit serving critically ill patients? The following "rough cut" figures (table 2) are based on my observations of what doctors actually do with the data provided.

Table 2. Tolerable error limits (± 2 standard deviations).

pH	± 0.02
Pco_2	± 3 mmHg at 40 mmHg
Po_2	± 5 mmHg at 100 mmHg
	± 3 mmHg at 60 mmHg
Base	± 2 mM/l

Do current instruments meet these minimum standards for accuracy and precision? Do current operators of instruments meet these standards? The following tables show that there is more lab-to-lab variation in precision, utilizing the same instrument-model, than there is between models from different manufacturers.

Table 3 shows data for pH. Note the striking differences in precision for the same instrument in different laboratories. These data were obtained by Dr. M. Miller of General Diagnostics utilizing a new ampouled blood gas control which I describe in greater detail in my other paper at this symposium.

Table 3. pH triplicates on 16 days, different labs different machines.

Instrument	N	Mean	%RSD
165 a	48	7.366	5.1
165 b	48	7.389	2.0
BMS 3	48	7.386	2.8
ABL1 a	48	7.374	5.5
ABL1 b	48	7.336	1.3
313 a	48	7.361	8.5
313 b	15	7.380	6.0
513	48	7.374	2.2
Labs' mean		7.376	
Manufacturer		7.371	1.4
(RSD calculated on H ion conc. not pH)			

Table 4 shows the data for Pco_2. We see again the striking differences in precision (expressed as %RSD or percent relative standard deviation, *i.e.* S.D. expressed as a percent of the mean).

Table 4. Pco_2 triplicates on 16 days, different labs different machines.

Instrument	N	Mean	%RSD
165 a	48	37.6	7.4
165 b	48	39.5	2.8
BMS 3	48	33.8	6.4
ABL1 a	48	35.2	11.5
ABL1 b	48	43.8	3.5
313 a	48	39.0	13.2
3.3 b	15	40.1	1.0
513	48	41.3	3.2
Labs' mean		38.6	
Manufacturer		39.9	1.8

Table 5 shows data for Po_2. The same story is again evident. There is greater variation between labs utilizing the same model of an instrument than there is between different models made by different manufacturers.

Table 5. Po_2 triplicates on 16 days, different labs different machines.

Instrument	N	Mean	%RSD
165 a	48	120.4	11.3
165 b	48	102.6	3.0
BMS 3	48	109.3	3.2
ABL1 a	48	126.8	14.9
ABL1 b	12	120.8	4.4
313 a	48	109.9	7.9
313 b	15	102.8	1.6
513	48	100.0	1.6
Labs' mean		107.5	
Manufacturer		104.9	1.4

These data indicate to me that our problem is more a problem of quality control at individual sites than it is a problem with manufacturers, or with primary standards for pH and gases.

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EFFECTS OF THE LIQUID JUNCTION ON pH MEASUREMENT IN BLOOD; THE 0.160 MOL/L SODIUM CHLORIDE BRIDGE

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1. Saturated KCl Bridge

The practical pH-cell is built up of an electrode sensitive to hydrogen ions (usually the glass electrode) and a reference electrode independent of the test solution (usually a calomel electrode) with a salt bridge of saturated KCl, which means that buffers and test solutions are applied in cells with a diffusion potential. Consequently the pH of a solution X is defined operationally by the relationship:

$$pH(X) = pH(S) + \frac{(E_X - E_S)F}{RT \ln 10} \qquad (1)$$

In most cases the liquid junction potential during measurement of the standard solutions is not equal to that existing during measurement of the test sample, that is, the residual liquid junction potential will be unequal to zero, with the result that the measured pH of the sample will hardly ever be exactly on the conventional pH(S) scale of the National Bureau of Standards (NBS). The degree of error can be calculated semiquantitatively from the molar conductivity and the concentration of the ions using Henderson's equation for the liquid junction potential between two solutions of different composition. With this formula we calculated some diffusion potentials of the junction: test soln. || satd. KCl at 25 °C [1][1].

Table 1. Calculated liquid junction potentials and residual liquid junction potentials of junctions with the saturated KCl bridge (see text).

No.	Solution X	I	Liquid junction potential mV	Liquid junction potential pH units	Residual liquid junction potential difference	Residual liquid junction potential mV	Residual liquid junction potential pH units
1.	equimolal phosphate buffer	0.100	2.1	0.035	1-2	0.1	0.002
2.	blood phosphate buffer	0.100	2.0	0.033	2-3	0.9	0.015
3.	0.160 mol/l NaCl	0.160	1.1	0.018	2-4	0.8	0.013
4.	0.025 mol/l $NaHCO_3$ + 0.135 mol/l NaCl	0.160	1.2	0.020	2-5	0.8	0.013
5.	liquor cerebrospinalis (CSF)	0.160	1.2	0.020	2-6	0.5	0.008
6.	plasma	0.160	1.5	0.025			

[1]Figures in brackets indicate the literature references at the end of this paper.

This table shows that:

a. A saturated KCl bridge provides junction potentials that are small: those of the phosphate buffers of NBS are nearly equal as well as those of spinal fluid (CSF) and plasma.

b. The residual liquid junction potential in the pH determination of CSF and plasma after calibration with the phosphate buffers is about 0.01 pH unit.

Various factors influence the liquid junction potential [1,2]:

a. Composition. Saturated KCl solution is mostly used as the bridge solution. Attention ought to be paid to dilution of the bridge solution, which gives lower results. So the pH of plasma is measured 0.003 pH lower with a 3.5 mol/l KCl bridge as recommended by Bates [3]. With a 0.15 mol/l KCl or 0.16 mol/l NaCl bridge, as we will discuss further on, values more than 0.1 pH lower than those using a saturated KCl bridge in the cell are obtained. Therefore, it is important to recommend the pH determination with the saturated KCl bridge as a reference method.

b. Structure. As demonstrated with the micro glass capillary electrode for over fifteen years, a stable and reproducible liquid junction is obtained when the test solution in a small plastic tube is dipped into the saturated KCl bridge solution, *i.e.*, the high density KCl solution is below the test solution. The opposite arrangement is unstable as the KCl tends to flow down. Allowance has to be made for this too when permeable membranes, separating the test solution from the KCl solution, are used at the boundary. As a consequence to such a bad construction, it is necessary to refill the reference electrode in the Corning M 165 blood gas instrument every day or two.

c. Temperature. We determined the change in the residual liquid junction potential with the temperature from the pH difference: pH (ref. 25 °C) - pH (ref. 38 °C) where pH (ref. 25 °C) and pH (ref. 38 °C) mean the values obtained with the same micro glass electrode at a constant temperature of 38 °C and two saturated calomel electrodes with a salt bridge, each at the indicated temperature. Both cells were calibrated with the equimolal phosphate buffer (pH = 6.840 at 38 °C). For blood, plasma, cerebrospinal fluid and a 0.025 mol/l sodium bicarbonate + 0.135 mol/l sodium chloride solution a difference of 0.011 pH was found; so, we calculate for the temperature coefficient of the residual liquid junction potential:

$$\Delta pH/\Delta t = 0.011/13 = 0.00085 \text{ pH/°C}.$$

The fact that this coefficient is equal for the mentioned solutions indicates that differences in activity and mobility of the potassium and chloride ions at 25 °C and at 38 °C are the most important factors. To reduce the influence of this thermal diffusion on the pH readings, the whole pH cell should be thermostated at one temperature, *i.e.*, 37 °C.

d. Effect of Blood Cells. To be able to understand in which order blood cells influence the diffusion potential of the salt bridge, we determined directly the liquid junction potential differences:

$$E_{j,p} \text{ (plasma } \| \text{ satd. KCl)} - E_{j,c} \text{ (plasma with cells } \| \text{ satd. KCl)},$$

which effect will from now on be called "cell effect."

We used the following cell:

Cal I, satd. KCl || plasma | suspension of cells in plasma || satd. KCl, Cal II

$E_{j,p}$ $E_{j,pc}$ $E_{j,c}$

If we put $\leftarrow_{j,pc}$ equal to zero, the emf difference will give the cell effect:

$$E = E_{\text{cal II}} + E_{j,c} - E_{j,p} - E_{\text{cal I}} \quad (E_{j,pc} \equiv 0).$$

We determined this effect at 25 °C and 38 °C. Figure 1 shows the cell effect in pH units of 10 experiments plotted as a function of the hematocrit.

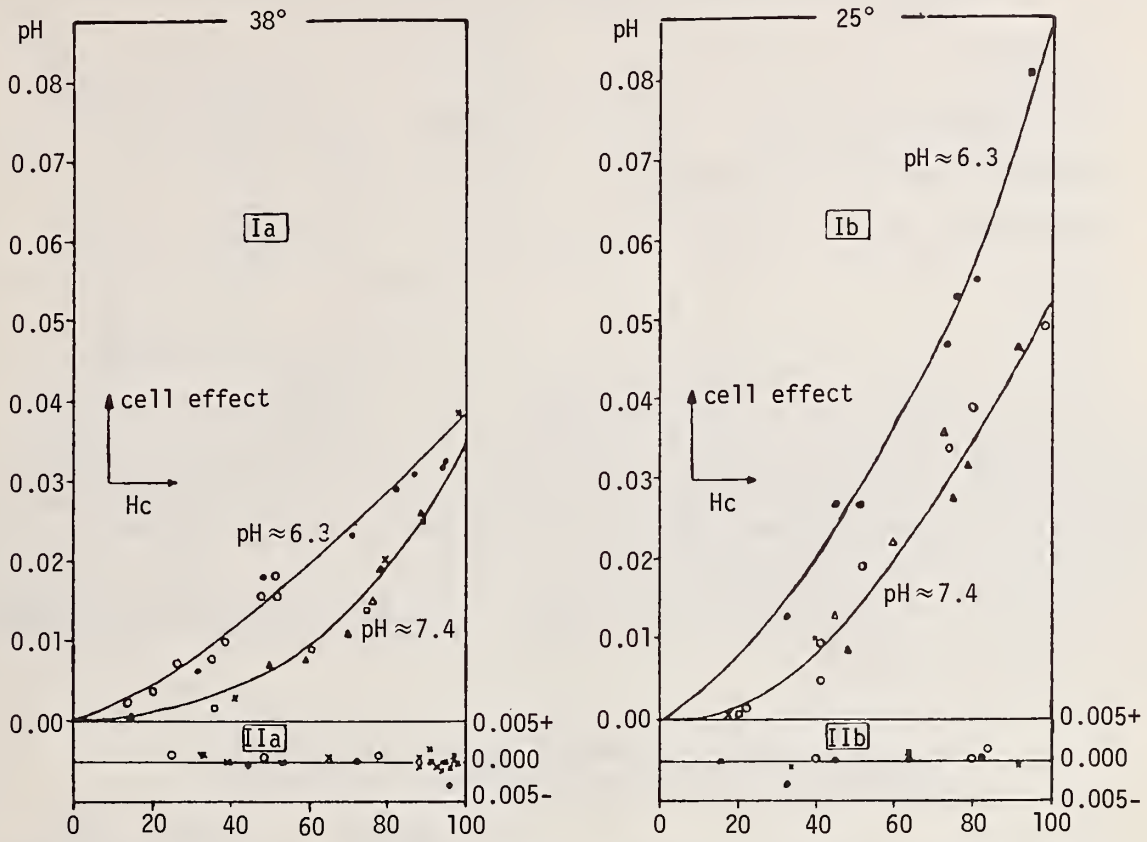

Figure 1. The cell effect at, respectively, the junction with saturated KCl (Ia and Ib) and with 0.160 NaCl (IIa and IIb) as a function of the hematocrit. The different symbols indicate different blood samples.

Graphs Ia and Ib show the cell effect increasing with the cell concentration. At lower pH, the cell effect is greater than at higher pH. At pH = 7.4 and normal hematocrit value (45%), the cell effect is 0.008 pH ± 0.002 (38 °C) and 0.012 pH + 0.002 (25 °C). To be able to establish whether the cell effect could be due to the negative charge of the erythrocytes (a so-called suspension effect), we determined in an analogous way the difference in diffusion potential of the bridge with an isotonic solution.

Graphs IIa and IIb show the cell effect of the 0.160 mol/l NaCl bridge plotted as a function of the hematocrit. We observe that even with hematocrit values of 90 percent, no cell effect exists. From the experiments we may conclude that the cell effect of the saturated KCl bridge is not caused by intact blood cells but most likely due to precipitation of proteins.

2. 0.160 mol/l NaCl Bridge

In order to avoid this disturbing effect of blood cells on the diffusion potential at the saturated KCl junction, we investigated whether the saturated KCl calomel electrode could be substituted by a calomel electrode with an isotonic salt bridge, notably the 0.160 mol/l NaCl bridge [4]. To that end, we made comparative pH measurements with the glass electrode-saturated KCl calomel electrode cell and the glass electrode - 0.160 mol/l NaCl calomel electrode cell; we calibrated with equimolal phosphate buffer.

The differences pH (satd. KCl) - pH (0.160 mol/l NaCl) were as follows:

Table 2. pH difference of values obtained with the glass electrode - saturated KCl calomel electrode cell and the glass electrode - 0.160 mol/l NaCl calomel electrode cell.

Solution X	pH range	pH (satd. KCl) - pH (0.160 mol/l NaCl)
Blood phosphate buffer	7.391	+ 0.005
0.025 mol/l $NaHCO_3$ + 0.135 mol/l NaCl	7.267	+ 0.153
Cerebrospinal fluid ("pooled")	7.486	+ 0.163
Plasma	7.453-7.481	+ 0.125 ± 0.005
Blood	7.311-7.370	+ 0.106 ± 0.005

This table shows that the pH of the blood phosphate buffer is influenced little. On the other hand, the pH values of body fluids measured with a cell with a 0.160 mol/l NaCl bridge, dependent on the test solution, are more than 0.1 pH lower than those determined using a cell with a saturated KCl bridge. Since the glass electrode, calomel electrode and standardization for both cells were the same, the pH differences must be interpreted as differences in residual diffusion potential.

This result will be more clear when we compare some diffusion potentials of the junction: solution X | 0.160 mol/l NaCl calculated with Henderson's formula. These values are given in the following table 3:

Table 3. Calculated and measured residual liquid junction potentials of junctions with 0.160 mol/l NaCl bridge

			Liquid junction potential			Residual liquid-junction potential			
							calculated		measured
No.	Solution X	I	mV	- pH units		diffe-rence	mV	pH units	pH units
1	equimolal phosphage buffer	0.100	8.1	0.137		1-2	1.1	0.019	0.005
2	blood phosphate buffer	0.100	7.0	0.118		1-3	7.1	0.120	0.153
3	0.025 mol/l $NaHCO_3$ + 0.135 mol/l NaCl	0.160	1.0	0.017		1-4	6.7	0.114	0.163
4	liquor cerebrospinalis	0.160	1.4	0.023		1-5	4.9	0.085	0.125
5	plasma	0.160	3.2	0.053					

This table shows that diffusion potentials of about 8 mV are found with the two phosphate buffers and much smaller values with body fluids and bicarbonate solution of I = 0.160. Further it appears that the calculated residual liquid junction potentials are of the same size as the measured differences. Just applying a pH cell with an isotonic salt bridge thus means, in the case of the 0.160 mol/l NaCl bridge, a shift in the pH scale of more than 0.1 pH with regard to the cell with a saturated KCl bridge, depending on the test solution. Both cells have to have the same pH scale if they will be useful. So new buffers had to be developed with NaCl added to limit the liquid junction potentials with regard to 0.160 mol/l NaCl. The pH value of these NaCl containing buffers can be determined in cells with a saturated KCl bridge.

Calibration buffers were prepared from the two standard phosphate buffers of the NBS. So much sodium chloride was added that the pH of solution X, measured with the cell containing the 0.160 mol/l NaCl bridge became identical with the pH measured with the cell containing the saturated KCl bridge. In our case, plasma was the solution to be measured. The following buffers appear to meet these conditions:

Table 4. pH(Ss,l.j.) values of phosphate buffers with NaCl at 25 °C and 38 °C.

Composition of buffers with NaCl	pH(Ss,l.j.) at 25 °C	pH(Ss,l.j.) at 38 °C
0.025 mol/l KH_2PO_4 0.025 mol/l Na_2HPO_4 0.130 mol/l NaCl	6.686	6.660
0.008695 mol/l KH_2PO_4 0.03043 mol/l Na_2HPO_4 0.120 mol/l NaCl	7.255	7.229

To agree with the pH(S) scale of NBS, we have attached an operational pH value (pH(Ss,l.j.)) to the standard buffers, which was measured with the hydrogen electrode-saturated KCl calomel electrode cell with regard to one of the NBS buffers. Redetermination by Drinker *et al.* [5] resulted in 0.01 pH lower values.

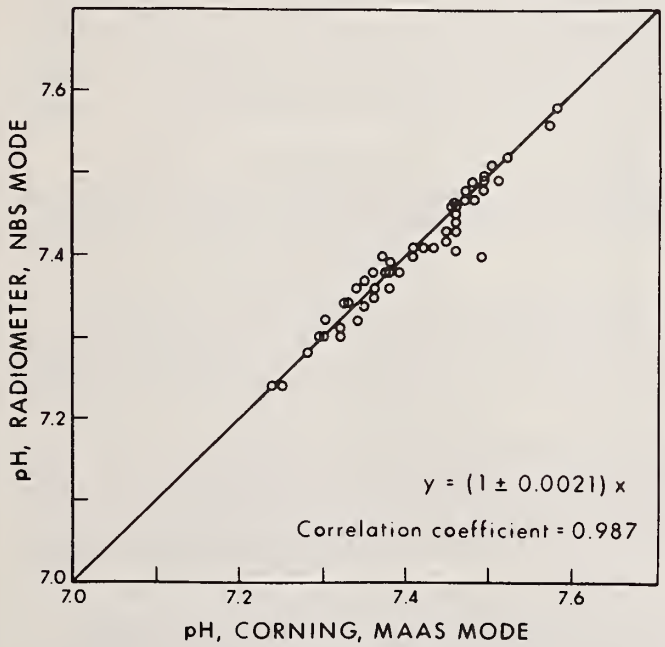

Figure 2. Comparison of blood pH measurements made with the Corning instrument operating in the Maas mode, and Radiometer used in NBS, or standard, mode.

For checking purposes, the pH's of a number of blood samples were measured and compared by means of the glass electrode-saturated calomel electrode and the glass electrode - 0.160 M NaCl calomel electrode cells. From the hematocrit value of the samples that pH determination with the cell with saturated KCl bridge was corrected for the cell effect. The average difference between the pH values of 21 blood samples obtained with both cells was -0.001 pH which, experimentally, was not significant. A glass electrode system with an isotonic salt bridge as used in this investigation, is completely reliable, and because of its special way of standardization perfectly equivalent to the conventional cells with a saturated KCl

bridge. Further, the pH cell with a 0.160 mol/l NaCl bridge offers the advantages that the liquid junction potential at the boundary between blood and 0.160 mol/l NaCl is independent of the concentration of blood cells and that it can be used without danger for continuous pH measurement in patients. Until now, this bridge was successfully applied by Drinker *et al.* using the Corning Model 165 instrument [5].

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THE CRITICAL CARE LABORATORY: A 10-YEAR PERSPECTIVE

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Fifteen years have passed since funds were first made available from the National Institutes of Health to the Department of Anesthesia at Massachusetts General Hospital for the evaluation of arterial oxygen partial pressure measurements as a clinical method for early detection of impaired postoperative blood-gas exchange. Progress achieved after a few years of endless frustration with methodology has been exceedingly rewarding. Although cause and effect are difficult to establish, one is tempted to conjecture that the birth of intensive care as a separate entity followed shortly after the arrival of the P_{O_2} electrode. The progress achieved from these early days characterized by endless frustration with methodology, have been exceedingly rewarding. Acute respiratory failure in the patient with previously normal lungs once considered a medical curiosity is now recognized as a national health problem and the routine measurement of arterial blood gases, particularly P_{O_2}, is an indispensable tool for early recognition, prevention, and therapy of abnormal lung function. An intensive care unit without access to blood gas measurement seems inconceivable today. Certainly, if an attempt is made to support patients with mechanical ventilation, lack of this facility may prove to be disastrous. An understanding of the growth and present-day problems attendant upon blood gas analyses can be gained by reviewing our experience for the past decade at the Massachusetts General Hospital.

In 1965, the importance of access to blood gases and pH for acute care was recognized by a greater number of our professional colleagues which, combined with the pressures imposed by growing clinical demands on a research facility, prompted formation of the Anesthesia Blood Gas Laboratory.

Remarkable foresight was exercised by the hospital administration and director of the Chemistry Laboratory (Dr. S. Rieder) when they insisted that the nature of the laboratory demanded supervision and control by physicians and/or personnel participating closely in the care of critically ill patients.

Figure 1 indicates how the demands upon the laboratory have evolved since its inception. By late 1975, we will have performed a grand total of 700,000 blood gas analyses, each one including P_{O_2}, P_{CO_2}, and pH. With the additional responsibilities listed in table 1, the laboratory now provides an average of 1049 analyses daily for 82 patients, all categorized as being critically ill.

These figures are not presented to impress. Rather, they go to the very heart of the problem we have gathered to discuss: (1) what are valid criteria for quality control, and (2) how is standardization of sensors and their calibration for every day use most appropriate?

Separation of ours from the main hospital laboratory was considered mandatory if a quick turn-around time was to be achieved. It was our original contention that a maximum period of 10 minutes be allowed for the time between arrival of the sample to the laboratory and a return telephone call to the patient's bedside with the answers. I regret to say that although we are not far away from the original limit, few requests find their way back within the ideal period. In the early days, problems with electrode technology were

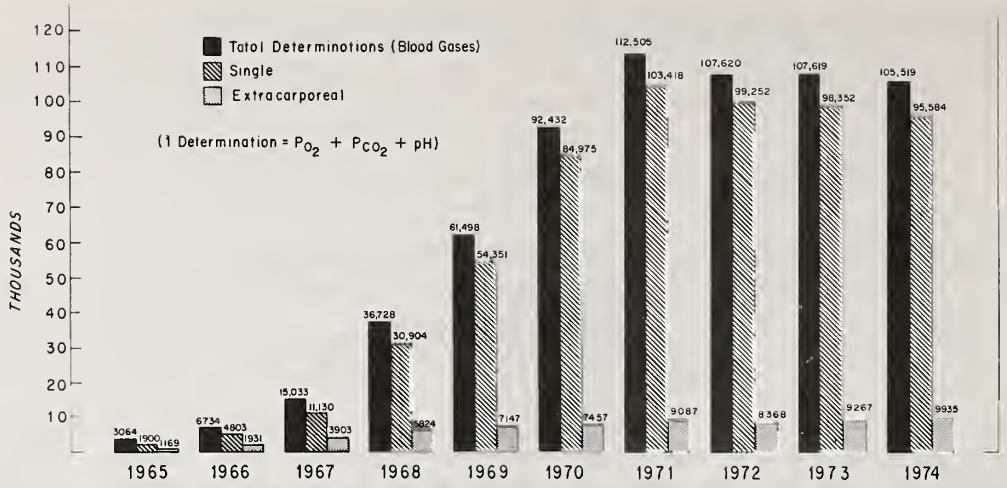

Figure 1. Yearly number of blood gas analyses performed in the Critical Care Laboratory of the Massachusetts General Hospital since its inception in 1965.

Table 1. Daily load in the Acute Care Laboratory.

Average number of patients studied/day = 82

Average number of individual analyses/day = 1049

Analyses:		
	PO_2 + Pco_2 + pH	Serum Osmolarity
	O_2 content	Urine Osmolarity
	Serum [K^+], [Na^+]	Total Protein
	Serum [Ca^{++}]	Cardiac Output
	Urine [K^+], [Na^+]	
	Hematocrit	

Total determinations: 10

Average number of analyses/open heart patients/day = 38

the principle reason. Today, we are overwhelmed by sheer numbers and delay is usually caused by our administrative inability to process the numerous samples pre- and post-analysis.

The laboratory is located adjacent to the Operating Room, the Recovery Room, the Respiratory Unit and the Surgical Intensive Care area. Unfortunately, the demand from other parts of the hospital is increasing as the incidence of acute respiratory disturbances is being recognized with greater frequency. Personnel coverage is shown in table 2. Twenty percent of the total technician time is spent in handling administrative detail unrelated to the measurements. The sensors have changed little while improvement in calibration, sample handling, and display of data has been significant (fig. 2).

Table 2. Personnel deployment.

	Hours	Number of Technicians	Technician-Hours/Day
Monday	7-3	5	40
to	3-11	4	32
Friday	11-7	3	24
			96
Saturday	7-3	3	24
and	3-11	3	24
Sunday	11-7	3	24
			72

Total technician-hours/week = 624

Average number samples/week = 1049 x 7 = 7343

Average number samples/technician-hours = 7343 = 11.8

A technician performs 1 analysis every 5.1 minutes.

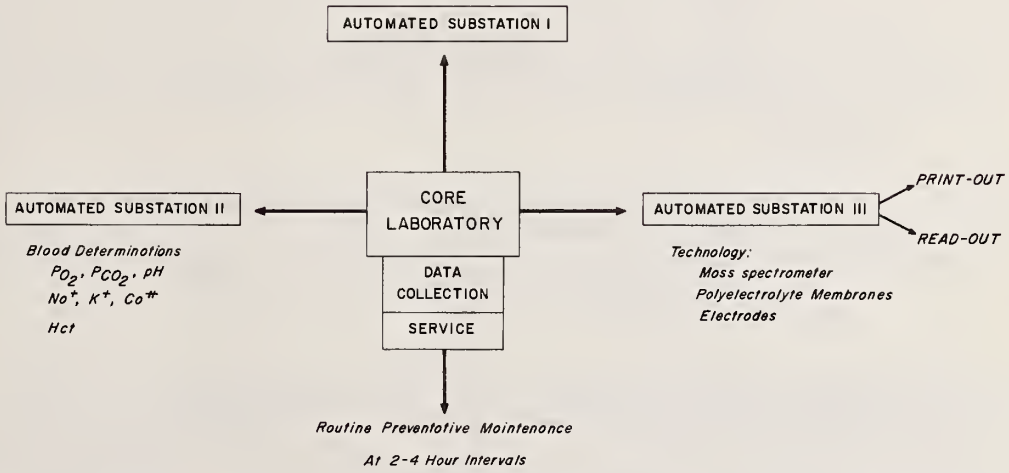

Figure 2. Potential plan for a decentralized automated critical care laboratory. The substations will be tied in with a core laboratory where the data are collected and stored. Errors and diagnostics are recorded continuously in the core laboratory from the substations.

Present problems include: (1) lesser control over individual technician performance, (2) prolongation of the turn-around time, and (3) excessive loss of personnel hours when samples are hand carried from distant parts of the hospital.[1] Points 2 and 3 suggest that

[1] We have calculated that approximately 6 miles are logged daily to hand carry samples from the bedside or every labyrinth of the hospital to the laboratory. If one includes the return trip, then the daily total is closer to 12 miles.

decentralization within this system must be considered. Together with a diminishing supply of motivated personnel these problems have emphasized the need for automation which will permit a short turn-around time assuming no loss of quality control. In order to improve our perspective for standards of accuracy we will review briefly the logistics that go into determining performance of an acute care laboratory. They apply equally well when attempting to improve an existing service (see table 3).

Table 3. Logistics to be considered in establishing a critical care laboratory.

1. Geography

Where is the laboratory to be located in relation to ICU, recovery room and operating room?

Will the demands of other parts of the hospital be met?

2. Turn-around time

How long after a blood sample is drawn does the answer return to the bedside?

3. Quality control

("Are the results trustworthy?")

Instrumentation:

1. How often are the delays prompted by equipment failure?

2. Is the service available from the manufacturer adequate?

Personnel:

1. Is training adequate to recognize and check for "bizarre" values?

2. Is there sufficient back-up to cover sickness and vacation?

4. Cost

How much and how often should the patient be charged?

1. Geography

Geography, *i.e.*, location in relation to the areas that need the service most. In the smaller community hospital, where separation of services is less than extreme, a decision as to location may be made with ease. In the larger institution such as a regional center where building plans generally perpetuate architectural and administrative obsolescence, centralization may be undesirable, if not impossible.

2. Turn-Around Time

Turn-around time, *i.e.*, the time elapsed between withdrawal of the blood sample and return of the results to the bedside. If the samples are hand carried by non-physician and/or non-committed personnel, the possibility of breakage and loss must be considered. If the laboratory facilities are strained (*e.g.*, numerous analyses requested at one time), then a delay is inevitable even if the sample arrives promptly in the laboratory.

3. Quality Control

Quality control, *i.e.*, consistent "trustworthiness" is the most difficult goal of all to achieve. Electrode technology is still an art, not science. In fact, the weak link is still the membrane-covered O_2 cathode. Although we have experienced significant qualitative improvement in recent years, the overall caliber depends on the experience of the technicians and their willingness to provide the extra effort necessary for frequent and appropriate calibration. Unfortunately, the gap between corporate profit motive and desire for excellence is still wide. Opinion is far from unanimous on the manner of calibration and many laboratories will modify the manufacturer's instructions to suit local needs. There is little reason left to continue P_{O_2} electrode calibration with liquids that provide readings equal to blood. The error introduced by using gas-equilibrated water, and in some systems, gas alone introduces errors that are of little clinical importance. Few will argue that for clinical purposes, a P_{O_2} error of 5 percent above 150 torr is unacceptable although improvement on this figure is most desirable. Because of the importance of obtaining a prompt answer, improvement in performance cannot ignore speed. Any device that does not allow a technician to process 15 samples per hour is unlikely to prove useful.

4. Cost

Cost has received little attention in the past. Our economy no longer permits such luxuries. Most laboratories have followed the pattern established for other services: one determination, one charge. If we agree that the acute phase of respiratory failure requires multiple blood gas analyses at short intervals, then the cost to the critically ill individual may reach astronomical proportions. One must consider the possibility that excessive cost will encourage a reduction in tests ordered. In order to prevent this trend, our laboratory initially instituted the "three unit charge" principle, or a maximum 24-hour charge per patient calculated on the basis of the cost for 3 analyses. All tests in excess of 3 were free of charge and physicians were encouraged to take samples as often as required by the patient's condition. In fact, it is not unusual for a patient in severe respiratory failure to have arterial blood samples drawn and analyzed at 15 to 30 minute intervals. Clinical experience suggests that this approach can go a long way toward prevention of catastrophies.

Because of the growing complexities in accounting, we have now established a fixed per patient per 24-hour cost with no limitation on the number of analyses performed. It may appear surprising, but effective administration makes this service quite equitable.

Finally, a comment on *in vitro* versus the *in vivo* blood gas monitoring.

When our laboratory first became a hospital service, a commitment had to be made regarding the type of monitoring most suitable for a large hospital population subject to a high incidence of respiratory failure. The intermittent route was chosen for four reasons. First, it permitted better quality control. Second, it allowed all services to take advantage of this facility. Thus, we did not restrict our services to the intensive care areas. Third, maintenance and fail-safe technology remained the responsibility of a small core of trained technicians. Fourth, it was more economical. Nothing that has transpired so far has given reason for a change in attitude.

5. Future

Reponsibilities of the Critical Care Laboratory have grown. As a result, its efficiency has suffered. It is fair to say that we now face two problems, both related to stability: the membrane-covered P_{O_2} electrode and personnel. The latter can be partially solved by a reduction in numbers, but not without increased automation. In fact, increased automation and decentralization are the directions for the future. Ultimately, automated substations will be located at several sites in the hospital. The problems there will be the same as we face today and faced ten years ago: formation of clot and electrode drift. Ancillary methodology requires critical evaluation. Mass spectrometry, modifications of

gas chromatography, dielectric dispersion spectrometry[2] deserve serious consideration to overcome the electrode drift problem. In the meanwhile, blood gas electrodes will be with us for years to come. The core laboratory will provide around-the-clock preventive maintenance service with the option of assuming responsibility for analyses if the substation is down. Details of substation design must incorporate the "building block" approach to facilitate replacement of malfunctioning parts. Although this may appear as an enormous task, I regard it as most feasible and even deserving of grant support.

It would be helpful to predict that our ability to prevent acute respiratory failure has advanced sufficiently to obviate an investment into a critical care laboratory. There is nothing in the natural history of the disease to suggest such a course. In fact, trauma and advanced heart disease are responsible for the most frequent causes of acute respiratory failure that require the services of a critical care laboratory. Unfortunately, "inadequate gas exchange" is easy to recognize while "adequate gas exchange" is a difficult diagnosis to make. Until such time that this statement will be obsolete, it is not likely that we will be out of business.

Dr. Henry K. Beecher, Professor of Anesthesia, Emeritus, gave the senior author unflinching support in the early days when blood gas electrodes and respiratory care were in their infancy; Miss Anna Murphy proved to be a loyal and critical assistant whose administrative devotion made the Critical Care Laboratory a reality. Finally, a laboratory working under pressure can only survive if both interest and morale are maintained at high level. We have been fortunate to receive superb support from numerous technicians. Although not usually the recipients of accolades, it is they who have made this endeavor a success.

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[2] Dr. A. Michaels, Alza Research, Palo Alto, California, personal communication.

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A THEORETICAL AND PRACTICAL ANALYSIS OF P_{O_2} MICROELECTRODE BEHAVIOR: THE THREE-SHELL MODEL

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A study was undertaken to examine the behavior of membrane-covered microelectrodes as predicted by a three-dimensional diffusion model. The following parameters have been found to be of importance for accurate prediction of electrode performance: (1) cathode diameter, (2) electrolyte layer thickness and permeability, (3) membrane thickness and permeability, (4) temperature, and (5) sample description. The model does predict the previously observed variations in electrode sensitivity, its dependence upon temperature, and the discrepancy we have noted in P_{O_2} readings between gas, water, and blood samples. The influence of sample viscosity is also predicted accurately. The changes that occur within the membrane including their influence on overall electrode stability and sensitivity have been considered. The signal decay observed with the electrode in a microcuvette has been discussed as an extension of the information provided by the theoretical model. This has allowed us to consider also the manner in which cuvette design influences electrode performance.

1. Introduction

Polarographic oxygen electrodes which incorporate a small metal cathode have found wide applications for biophysical research and are now used routinely in the measurement of blood gases. When used *in vivo*, the electrodes allow for the measurement of P_{O_2} in flowing blood, in different tissues, and even at the cellular level [1-5][4]. *In vitro* applications include the analysis of oxygen partial pressure in the gas phase, liquids, and whole blood [2,3,6-8], the measurement of oxygen reaction rates in the Hartridge-Roughton fast-reaction apparatus [9], and finally, evaluation of tissue metabolic rates [10]. These applications are possible because the microelectrode consumes very little oxygen and membranes with high diffusivity for oxygen can be used to cover the electrode surface. The original purpose in using a covering membrane was to obviate cathode poisoning due to protein deposition [2]; however, an important function of these membranes is to isolate the oxygen diffusion field surrounding the cathode [3,4,11].

Proper performance of microelectrodes is dependent upon a stable and reproducible diffusion field [3,4]. Such a field is generally more difficult to attain then the potential fields required for thermodynamic electrodes, making the polarographic electrode more sensitive to artifact. The sensitivity (amps output/mm Hg P_{O_2}) is subject to constant drift and is not independent of the sample characteristics [12], *i.e.*, variations in hydrostatic pressure applied during injection of a sample and temperature, cause rapid and unpredictable variations in sensitivity. For example, the sensitivity for gas will be different than for liquid samples with the same oxygen tension (P_{O_2}) [6,11] stirred samples read differently from stagnant ones [12], and sample viscosity causes variations in sensitivity [1,13]. As a result of these complications, frequent calibration is mandatory and, depending on the accuracy desired, the use of tonometered fluids of the same con-

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[4]Figures in brackets indicate literature references at the end of this paper.

sistency as the sample has been recommended [12]. Because the latter approach is impossible with implanted electrodes, *in vivo* results are difficult to quantify [3].

Several mathematical models have been proposed to describe P_{O_2} microelectrode behavior [3,4,11,14-16]. However, none describe the behavior of the electrode even in a well-characterized *in vitro* environment. If a model is to be useful, it must predict quantitatively electrode sensitivity, the temperature dependence of sensitivity, and the influence of different membrane characteristics on cathode performance. Both the simple spherical [15] and linear diffusion models [3] predict a much lower sensitivity [11,12] and a greater temperature and membrane thickness dependence than observed experimentally [12].

The literature also indicates the electrode behavior depends on the environment in which it is used. Some authors have described a linear relationship between temperature and sensitivity [9]; according to others, this relationship is exponential [12].

There are reports that the sensitivity is greater in water than in air [11] and vice versa [6]. In small cuvettes, the sensitivity in liquid samples decreases with time and anomalous signals may be recorded during sample injection [12].

Because of inconsistencies in the literature, we reported earlier our studies with several commercially available cathodes maintained in a well-characterized environment *in vitro* [12]. We now describe a theoretical analysis of electrode behavior with numerical solution of the three-dimensional diffusion process. The latter is compared to the idealized models often used in biophysical modeling. The microelectrode serves as a prototype for a study of transmembrane transport problems and thereby exemplifies the influence of spatial geometry on the behavior of mathematical models dealing with diffusion.

A. Mathematical analysis

Figure 1 is a schematic presentation of an electrically isolated microelectrode. It consists of a fine wire (diameter < 0.050 mm) imbedded in an inert matrix (glass or epoxy),

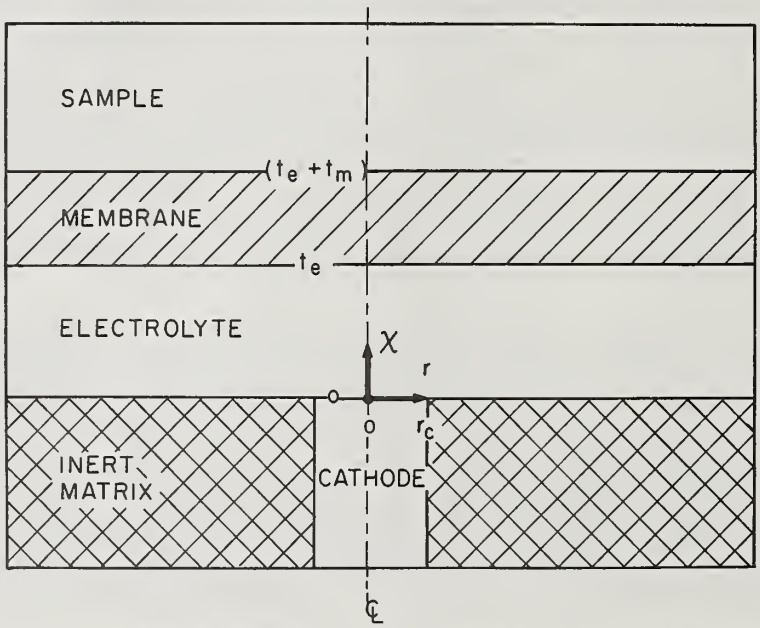

Figure 1. Simplified O_2 cathode geometry; r_c = cathode radius; r = lateral, radial distance away from cathode center; X = vertical distance from cathode surface; $t_e + t_m$ = sum of electrolyte and membrane thicknesses.

while its end remains exposed. The surface of the inert material may or may not be smooth as shown in the figure; most often it has a finite surface roughness that may reach a depth of 20 to 30 micrometers. This assures a continuous electrolyte bridge. Surface of

the cathode is also rounded but its radius of curvature is so much greater than the cathode diameter (the latter defines the size of the diffusional field) that the surface can be considered flat as in the vicinity of the cathode. If we accept the geometric simplification shown in figure 1, we can characterize the diffusion of oxygen from sample to cathode in terms of a cylindrical coordinate system. The sample whose P_{O_2} is being evaluated may be either a stagnant liquid or a well-defined segment of tissue. If the fluid is stirred, diffusion occurs only through a thin layer between sample bulk and membrane, where it is assumed that the liquid flows in laminar fashion over the membrane surface.

The diffusion of oxygen to the cathode is defined by the cylindrical coordinate system, using the center of the cathode surface as the origin [17]. For a stagnant sample, the differential equations describing the diffusion of oxygen from sample to cathode can be expressed as follows:

$$D_s \cdot \frac{1}{r} \cdot \frac{\partial}{\partial r}\left(\frac{r\partial P_s}{\partial r}\right) + \frac{\partial^2 P_s}{\partial X^2} = \frac{\partial P_s}{\partial t} + M \tag{1}$$

$$D_m \cdot \frac{1}{r} \cdot \frac{\partial}{\partial r}\left(\frac{\partial P_s}{\partial r}\right) + \frac{\partial^2 P_s}{X^2} = \frac{\partial P_m}{\partial t} \quad (t_e \leq X \leq (t_m + t_e) \tag{2}$$

$$D_e \cdot \frac{1}{r} \cdot \frac{\partial}{\partial r}\left(\frac{r\partial P_s}{\partial r}\right) + \frac{\partial^2 P_s}{\partial X^2} = \frac{\partial P_e}{\partial t} \quad (0 \leq X \leq t_e) \tag{3}$$

where D = diffusivity (cm^2/sec)

P = P_{O_2} (mm Hg)

M = O_2 metabolic rate divided by the O_2 solubility coefficient (mm Hg/sec)

e = electrolyte

m = membrane

s = sample

X = vertical distance from cathode

At a large distance away from the cathode, the oxygen gradient disappears (*i.e.*, $(\partial P/\partial r) = (\partial P/\partial X) = 0$); the gradient at the other boundaries defines the coupling of eqs. (1) through (3). Therefore,

$$\frac{\partial P_s}{\partial X} = 0 \quad (X \to \infty) \tag{4}$$

$$\frac{\partial P_s}{\partial r} = \frac{\partial P_m}{\partial r} = \frac{\partial P_e}{\partial r} = 0 \quad (r \to \infty) \tag{5}$$

$$D_{s\alpha s}\left(\frac{\partial P_s}{\partial X}\right) = D_{m\alpha m}\left(\frac{\partial P_m}{\partial X}\right) \quad X = t_m + t_e \tag{6}$$

$$P_s = P_m$$

$$D_{m\alpha m}\left(\frac{\partial P_m}{\partial X}\right) = D_{e\alpha e}\left(\frac{\partial P_e}{\partial X}\right) \quad X = t_e \tag{7}$$

$$P_m = P_e$$

$$kP_e = D_{e\alpha e}\left(\frac{\partial P_e}{\partial X}\right) \quad (X = 0,\ r \leq rc) \tag{8}$$

$$\frac{\partial P_e}{\partial X} = 0 \quad (X = 0,\ r > r_c) \tag{9}$$

where k = O_2 reduction rate at the cathode

r_c = radial distance from cathode center (fig. 4)

α = Bunsen solubility coefficient for O_2.

For the initial conditions we may assume that α is a constant concentration of oxygen present throughout the three regions, and at time zero the electrode is polarized. This can be analyzed with eqs. (1) through (9) to give the electrode current (i) as a function of time:

$$i = nD_e\alpha_e F \int_{X=0} Ac\left[\frac{\partial P_e}{\partial X}\right] dA_c \tag{10}$$

where n = number of electrons generated per O_2 molecules reduced

A_c = cathode surface area

F = Faraday's constant

It is commonly assumed that the reaction rate for oxygen at the cathode is infinitely fast when compared with the diffusion rate (in eq. (8), $k \simeq \infty$). Under these conditions, $P\ (x = 0) = 0.0$ and the O_2 reduction rate is limited by O_2 diffusion to the cathode [15]. However, the polarograms shown in figure 2, by the absence of a definite plateau, do not indicate any region of complete diffusional limitation, except perhaps at low oxygen tensions. Therefore, the rate of reaction is included for completeness. The equations presented here do not include initial conditions nor do they consider the fate of oxygen once it has reacted at the cathode.

If the electrode is polarized at time zero, the recorded current must include also the current due to ion migration in the electric field; the time required to establish a stable ionic electric field is greater than the diffusional response time and involves far greater currents than are attributable to oxygen reduction. Therefore, it is best if we assume a fully polarized and stabilized electrode whenever eqs. (1) through (9) are used to characterize cathode performance. The solution to the initial condition of constant P_{O_2} does indicate the response time to a change in P_{O_2} of the polarized electrode.

The "n" term in eq. (10) is normally assumed equal to 4.0. However, there is evidence that oxygen reduction to hydroxyl ions is not complete on a stoichiometric basis. Satterfield [18] has shown that H_2O_2 can diffuse away from the cathode and be converted back to oxygen. Under these conditions one cannot be certain that exactly 4 electrons correspond to one mole of oxygen reduction at the cathode (eq. (10)).

Although eqs. (1) through (3) are linear, the analytic solution is not easily obtained. Therefore, we have utilized the Method of Zones numerical technique for their solution [19]. Figure 3 presents the results of these computations, where cathode current and sensitivity are given as a function of time. We have assumed that the electrolyte, membrane, and sample are in equilibrium at a P_{O_2} of 100 mm Hg and the electrode reaction rate is infinitely fast. The sample volume is taken to be 1×10^{-10} ml[5] with an oxygen solu-

[5] This sample corresponds to a 20 μm layer (unstirred) with the same radial dimensions as selected for the membrane and electrolyte (see fig. 1). A larger sample volume is more complex, mathematically speaking, but would not alter materially the results.

bility coefficient of 0.0238 ml (STP)/ml-atm. The membrane is polyethylene, 25 micrometers thick with the permeation properties shown in figure 3 [20]. The electrolyte layer thickness is assumed to be 20 μm (see Sec. 2, Results) and with the same O_2 permeation properties as water.

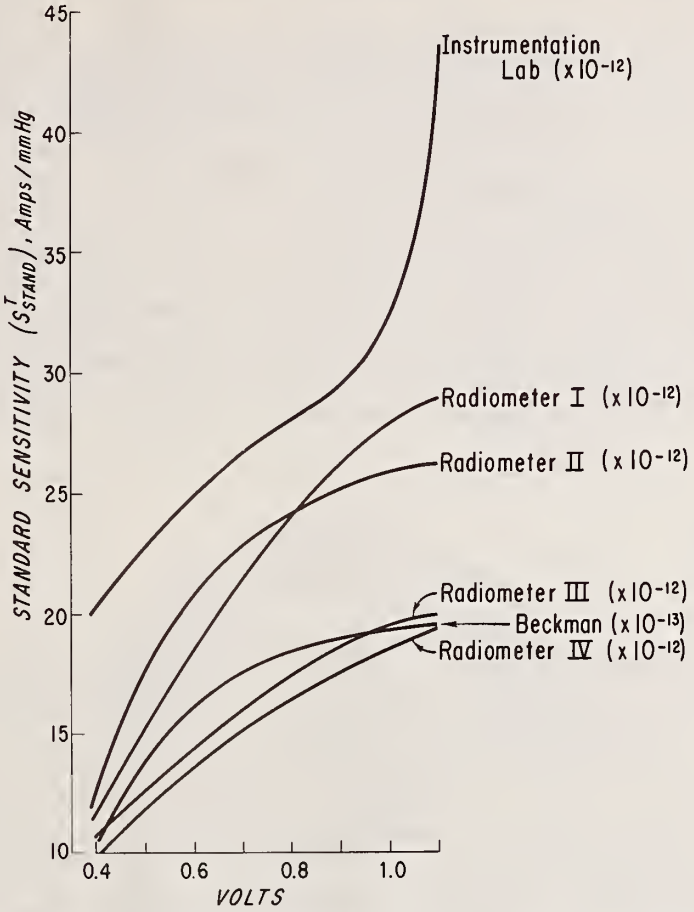

Figure 2. Effect of polarizing voltage on standard sensitivity (S^T_{STAND}) at P_{O_2} for ambient air of various commercially available oxygen electrodes. Sample: stirred water at 38 °C. Membrane: polypropylene 0.015 mm.

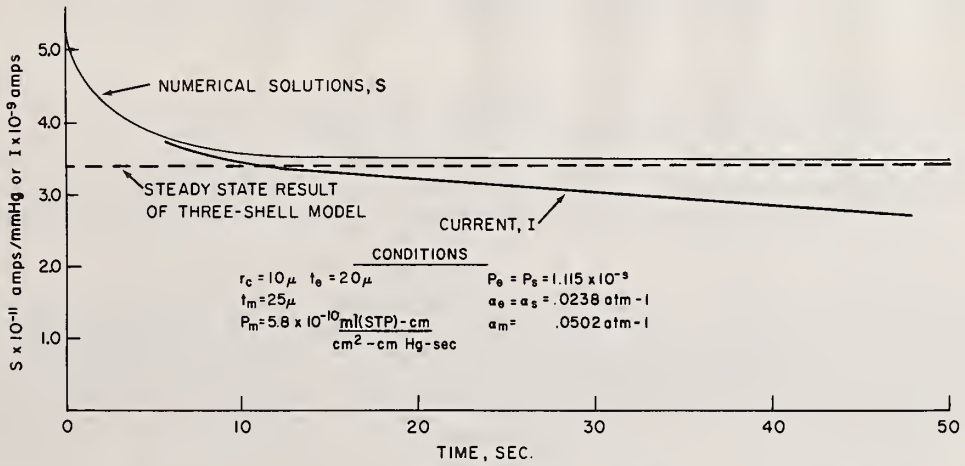

Figure 3. Electrode sensitivity (S), or the ratio of current (I) to sample P_{O_2} was plotted as a function of time. The sample P_{O_2} is the average value of the four-sample zones indicated in figure 4. For symbols see Appendix.

The current decreases rapidly for about 10 seconds, then levels off to decrease at a constant rate. During the rapidly decreasing portion of the curve, oxygen dissolved in the electrolyte and membrane (both at P_{O_2} = 100 mm Hg) is being consumed. Since oxygen from both sites diffuses a shorter distance than oxygen from the sample, the rate of arrival at the cathode surface is greatly increased. As this O_2 is consumed, the P_{O_2} near the cathode decreases, thereby establishing a gradient from the sample to the cathode. The current continues to decrease linearly because the sample size is too small to allow for a sufficient fraction of dissolved O_2 to diffuse to the cathode. Since this rate of decrease is linear, we must infer that the overall electrode response time (a complex function of the diffusive properties of electrolyte, membrane, and geometry of the system) is much less than the rate of oxygen depletion from the sample. Thus, although the sample P_{O_2} is decreasing continuously the concentration profile within the electrolyte and membrane readjusts so rapidly that the ratio of P_{O_2} remains constant with time.[6] Presence of this condition is supported by the graph which expresses electrode sensitivity (S) as a function of time (fig. 3). After the initial transient period, sensitivity appears to be independent of time (and therefore, P_{O_2}). The time constant for the appearance of a stable sensitivity is 2.8 seconds, while the predicted time constant for linear diffusion through the membrane (*i.e.*, t_2/D_m) is 7.1 seconds. The fact that the sensitivity does pass through a transient period is contrary to the predictions of Hudson [14]. The steady state isobars for the electrode which we described in figure 3, are shown in figure 4. The lines are seen to

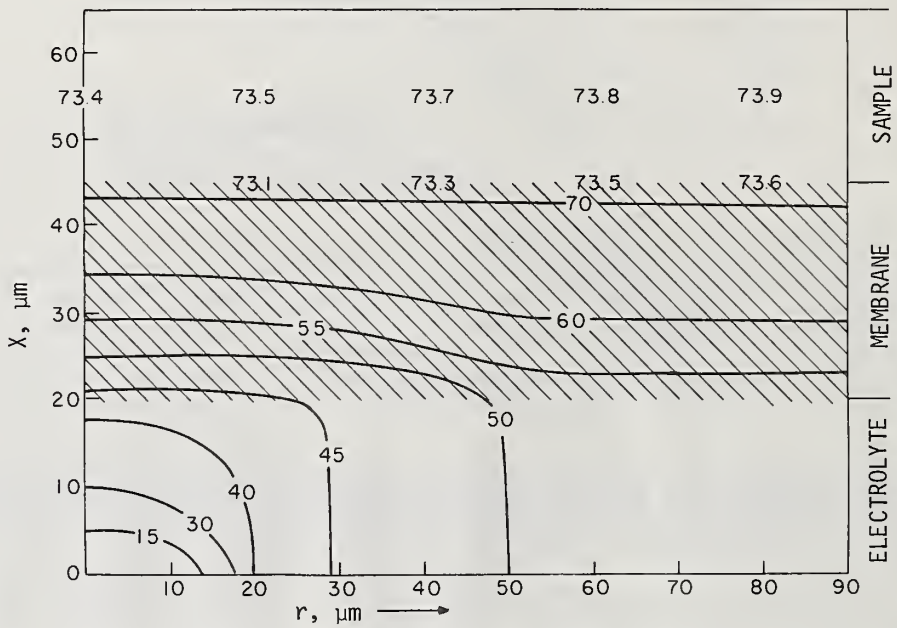

Figure 4. P_{O_2} isobars surrounding the cathode under steady state conditions. These values are by numerical solution of eqs. (1) to (9). This consists of dividing each zone (electrolyte, membrane, and sample) into four radially-located zones and several zones in the X direction. O_2 flux equations for each zone are derived from eqs. (1) to (3) and solved simultaneously by using the boundary conditions described in eqs. (4) and (9). Note in these considerations, the sample P_{O_2} has decreased from 100 to 73.8 mm Hg.

form an approximately spherical surface around the cathode. P_{O_2} in the electrolyte at a radial distance of 90 μm, or 9 times the cathode radius (r_c = 10 μm) is less than the sample P_{O_2} while P_{O_2} at the membrane-sample interface is within 0.6 percent of the sample value. By computing the P_{O_2} gradient in the electrolyte and membrane from these isobars, one can see that over 98 percent of the oxygen flux comes by diffusion in a radial direction within the electrolyte (*i.e.*, it arrives by crossing the membrane in the region where $r > r_c$). If the electrolyte layer were thinner, this flux would not be significant.

[6]For a more detailed analysis of concentration-relaxation problems, see reference [21].

However, because of the large radial flux term, the amount of oxygen reaching the edge of the cathode is greater than that which reaches its middle. Thus, the electrolyte layer thickness (ELT) is an important determinant of total flux because an increase in ELT permits oxygen transport across a larger membrane area and results in a higher measured electrode sensitivity.

From these studies of diffusion patterns in the peri-electrode space, it became apparent that a geometrically simpler model would provide a useful tool in our attempt to account for the influence of ELT on electrode performance. In the spherical model shown in figure 5,

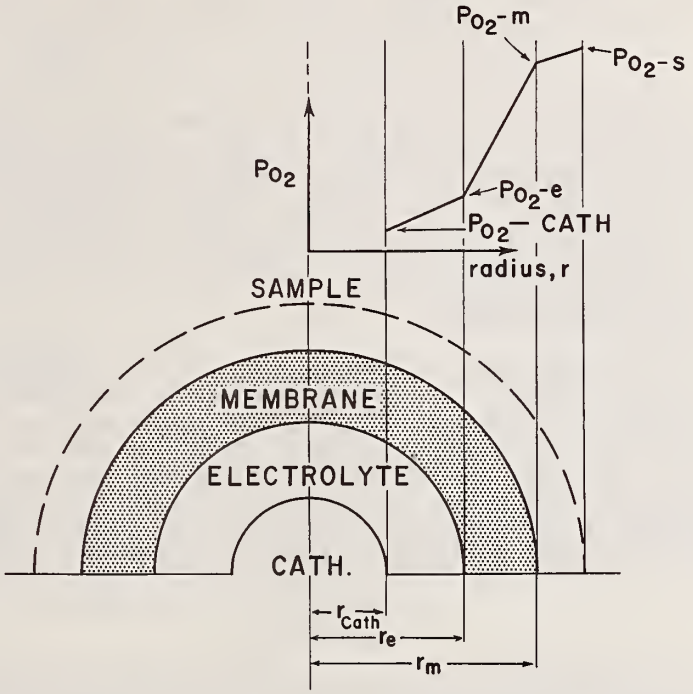

Figure 5. Idealized 3-zone model of the oxygen microelectrode, electrolyte, and membrane complex. The radial P_{O_2} gradient under steady state conditions assumes a significant resistance in each zone. For symbols see Appendix.

the cathode is treated as a hemisphere surrounded by hemispherical shells of electrolyte and membrane. The actual cathode radius is used but the current (i), or

$$(\frac{2}{3}\, n\, F\pi r^2 c\, \frac{\partial P}{\partial r})r_c,$$

is defined in terms of a spherical area on the assumption that oxygen flux density is uniform over this cathode. An increase in area compensates for the increased flux density near the outer edge of the cathode as determined in the previous model and, by increasing the ELT, the effective membrane is also increased, thereby increasing sensitivity. We have used the model only to predict the steady-state electrode sensitivity because this is most readily shown for a spherical model [15]. The oxygen transfer rate from sample to membrane surface is characterized by a transfer coefficient which can be expressed as a function of stirring rate. The same boundary equations apply as given in eqs. (4), (5), (7), and (8). While the boundary conditions expressed by eq. (6) are replaced by:

$$D_{m\alpha m}\left(\frac{\partial P_m}{\partial r}\right) = k(P_s - P_m) \qquad r = r_m. \tag{11}$$

The mass transfer coefficient, k, is used to allow for the effect of stirring. Also, since convection is never absent in the cuvette chamber (particularly when one is using whole blood), use of eq. (6) provides only an approximation. As there are several empirical correlations available for predicting k, its incorporation into the model does not seriously limit the model's utility. (See Appendix for a description of one method for predicting k.)

The steady-state solution for sensitivity (S) in the spherical 3-shell model is given by:

$$S = \frac{i}{P_s} = \frac{2\pi nF}{22{,}400} \Big/ \left[\frac{1}{kr^2_m} + \frac{t_m}{P_m r_m r_e} + \frac{t_e}{P_e r_e r_c} \right] \qquad (12)$$

where P_m is the product of D and α, the so-called permeability coefficient. Using the same constants as were used in the numerical solution presented in figures 3 and 4, the 3-shell model predicts a sensitivity (S) of 3.38×10^{-11} amps/mm Hg, while the steady-state value from figure 3 is 3.45×10^{-11} amps/mm Hg. If one uses the simpler spherical model (without an electrolyte layer), the predicted sensitivity will be 8.8×10^{-12} amps/mm Hg, while the linear diffusion model [3] predicts a sensitivity of 3.15×10^{-15} amps/mm Hg. The 3-shell model appears to agree favorably with the numerical solution, while the simpler models predict a much lower cathode sensitivity.

Because of the reasonably good agreement we have found between the numerical solution and the 3-shell model, we elected to use this more easily manipulated model for our evaluation of electrode behavior. Equation (11) indicates the properties of sample, membrane and electrolyte required to evaluate the sensitivity of the electrode. Information required include cathode size, membrane thickness, ELT, temperature, membrane and electrolyte permeability, and finally, sample characteristics. Since ELT is usually not known, all other information must be available in order to infer its value. The results to be reported indicate that all these parameters are indeed significant.

2. Results

Using data published by others and our own [12], we have calculated: (a) electrode sensitivity, (b) temperature-dependence of the sensitivity, and (c) dependence of sensitivity on sample type. A technique for evaluation of the influence of electrolyte layer thickness is also described.

A. Electrode sensitivity

Prediction of electrode sensitivity requires all the information described in eq. (12). Unfortunately, there are no reliable methods at present for determining the electrolyte layer thickness directly, therefore its value must be inferred. Failure to include the electrolyte layer thickness has resulted in models that do not satisfy the observed electrode behavior. Figures 6 and 7 indicate the dependence of electrode sensitivity on electrolyte layer thickness for two different membranes and several membrane thicknesses. The sensitivity increases with increasing electrolyte layer thickness because the high electrolyte permeability (table 1) does not limit oxygen diffusion so as to offset the increased area

$$[A = \tfrac{2}{3}\pi(r_{CATH} + t_e + t_m)^2]$$

on the outside of the membrane. For each membrane thickness, several curves are drawn, the lower one for stagnant water and the upper curve for a gas sample.

In figure 6 we have plotted our experimental data for two values of membrane thickness on lines calculated according to eq. (12). These lines indicate the values for sensitivity when the electrodes record P_{O_2} in air, stirred water, and stagnant water. For each membrane

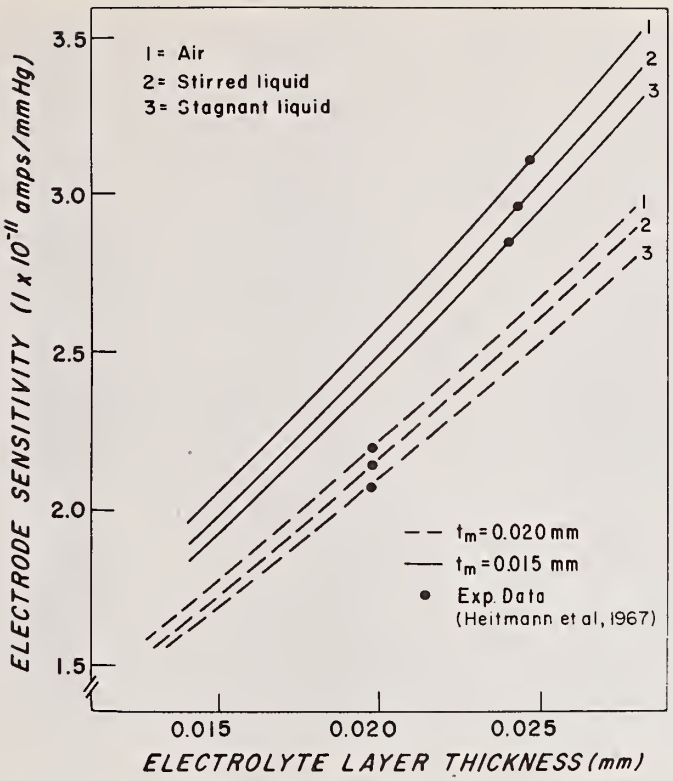

Figure 6. Electrode sensitivity as a function of electrolyte layer thickness for polypropylene membranes of different thicknesses. The curves are calculated with the 3-zone model, *i.e.*, eq. (12), based on the following conditions: T = 38 °C; cathode diameter, 0.020 mm; sample, water equilibrated with ambient air.

thickness, the three data points obtained with a microelectrode predict the same electrolyte layer thickness, irrespective of sample type. The electrolyte thickness so determined for several electrodes covered with polypropylene fell in the range of 0.020 to 0.030 mm. It is important to note that a change in sensitivity caused by a change in electrolyte layer thickness does not alter the difference between the readings obtained with gas and stagnant liquid sample.

Similar data have plotted for two different thicknesses of Teflon FEP membranes at 38 and 25 °C (fig. 7). The derived range of electrolyte thickness is 0.0055 to 0.007 mm. In figure 8 we have indicated the sensitivity dependence on cathode size for a 0.015 mm polypropylene membrane at 38 °C using the derived electrolyte thickness of 0.025 mm.

In an attempt to measure directly the influence of electrolyte layer thickness on electrode sensitivity, we constructed the device (*i.e.*, electrode holder) shown in figure 9. The micrometer permits adjustment of the cathode tip distance (any commercially available microelectrode can be used) relative to the fixed membrane in steps of several micrometers. The hydrostatic pressure of the electrolyte is maintained constant by an overflow hole. A sequence of runs were made by suspending the electrode in a thermostatted bottle filled with water, stirred by a magnetic bar at 300 rpm, and through which we bubbled a gas of known oxygen concentration. The cathode was positioned at about 0.75 mm above the membrane and a steady state reading obtained. The micrometer was then turned to the next unit (0.032 mm/mark) and the new sensitivity determined. This procedure was continued until the membrane appeared to be visibly distended.

Figure 10 presents the results of these measurements. Two extreme cases may be estimated by simplified models and the theoretical maximum and minimum sensitivity computed. Maximum sensitivity (S_{MAX}) must occur at an electrolyte layer thickness where the membrane no longer influences the diffusion field. In this case, the electrolyte will be equilibrated with the sample P_{O_2} and the spherical-diffusion-in-a-homogeneous-medium model

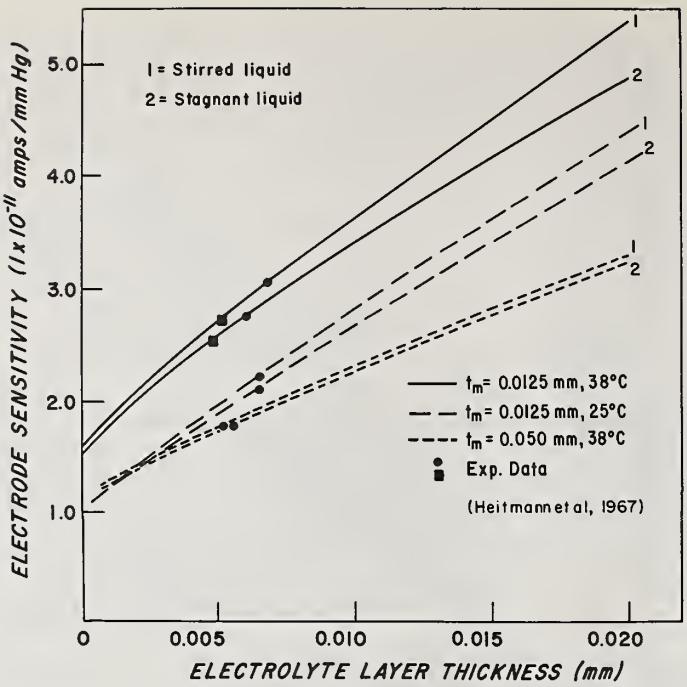

Figure 7. Electrode sensitivity as a function of electrolyte layer thickness for different thicknesses of Teflon FEP membranes. The curves calculated with the 3-zone model based on the following conditions: T = 38 or 25 °C, as indicated; cathode diameter, 0.020 mm; sample, water in equilibrium with ambient air, stirred or stagnant; membrane thicknesses from 0.0125 to 0.050 mm as indicated. Experimental data were obtained from Heitmann *et al.* [12]. Note that Teflon FEP (0.050 mm) exhibited the smallest theoretical difference between stirred and stagnant water; experimentally no significant difference was found between these two samples.

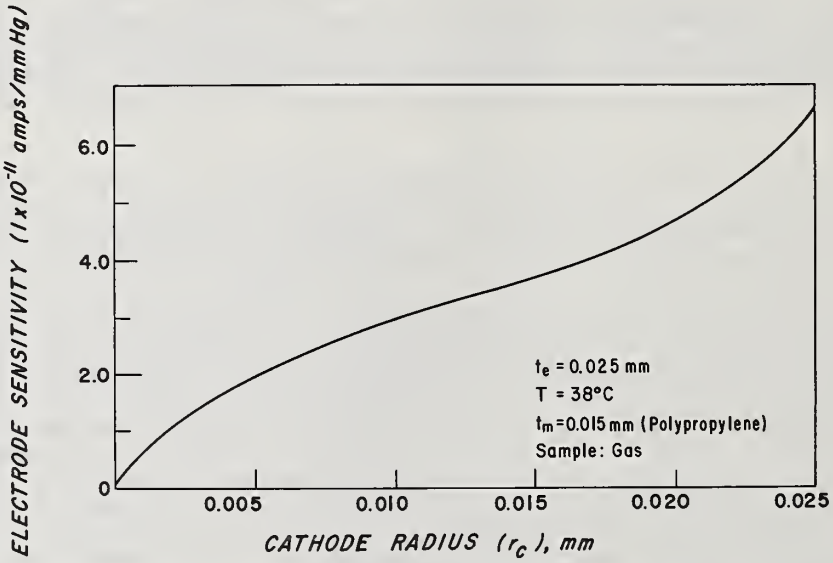

Figure 8. Dependence of electrode sensitivity on cathode size under the conditions stated.

should apply [15]. This value is plotted in figure 10 and is about the same as the maximum we have obtained experimentally. Minimum sensitivity (S_{MIN}) will be obtained when the spherical diffusion flux is totally restricted to the membrane; *i.e.*, when the electrolyte

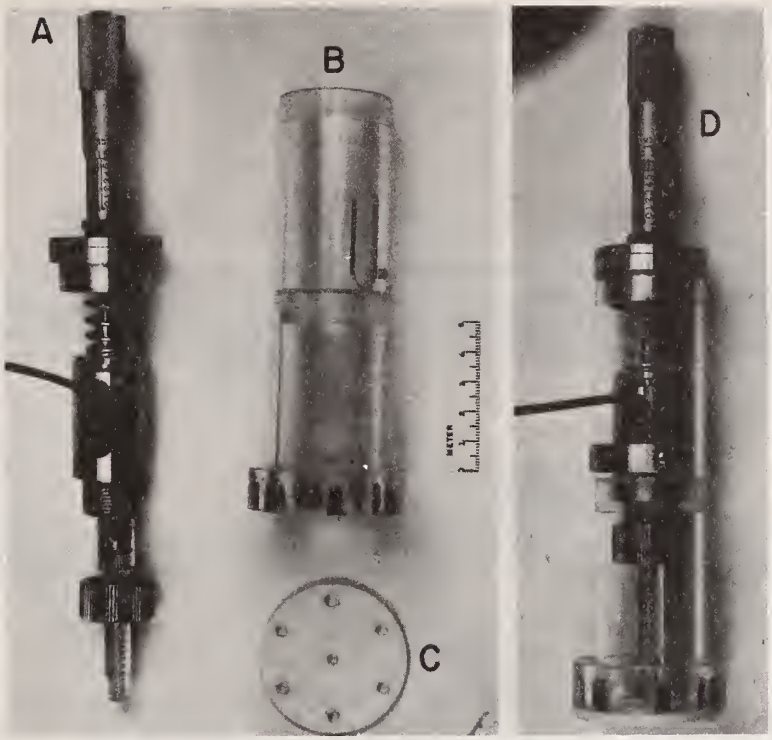

Figure 9. Cuvette-electrode assembly which permits a change in electrolyte layer thickness. A. Electrode-micrometer assembly which allows for an accurate displacement of the electrode away or toward the membrane. B. Housing. The electrolyte is contained within the lower half of the plexiglas chamber. C. Membrane holder plate. D. Assembled electrode.

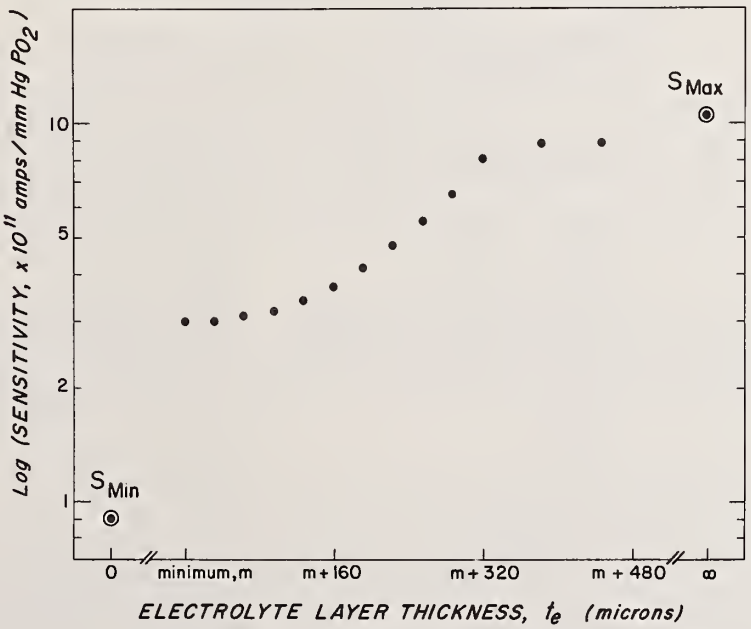

Figure 10. Influence of electrolyte layer thickness (ELT)(t_e) on electrode sensitivity (S). Maximum sensitivity (S_{MAX}) is recorded at right (◉) at an electrolyte layer thickness which is large enough so that the membrane no longer influences the diffusion field. Point at left (◉) indicates the theoretical minimum sensitivity (S_{MIN}). At S_{MAX}, behavior of the electrode is membrane-independent and ELT dependent; conversely, at S_{MIN} electrode behavior is limited principally by membrane diffusivity and solubility for O_2 and less by these properties in the electrolyte.

layer thickness is zero. The theoretical value, also plotted in figure 10, is much lower than the actually determined minimum sensitivity. The asymptotic approach to the experimental minimum sensitivity suggests that one can never reach the level of a zero electrolyte layer thickness. These results suggest that the electrolyte layer thickness in responsible for the changes in sensitivity of microelectrodes over the span of theoretical values used.

B. Temperature dependence of electrode sensitivity

According to eq. (12), three temperature-dependent parameters determine electrode sensitivity. They are: permeability of the electrolyte, membrane, and sample. It can be shown that one need only consider behavior of the electrolyte layer and membrane in order to determine the true electrode temperature sensitivity. Both of these parameters are given by the general form:

$$P_i^T = \overline{P}_e^{-\Delta E/RT} \tag{13}$$

where $\overline{P}$ and E_p are obtained from table 1. Within the limits of 0 to 50 °C, one can also express the temperature dependence of electrode sensitivity in the following form:

$$S^T = \overline{S}_e^{-\beta/RT} \tag{14}$$

where P_i^T is obtained from table 1. Within the limits of 0 to 50 °C, one can also tionality. The rate of change of S^T with respect to the temperature, T, is not constant; rather, it decreases with increasing temperature. Thus, it is preferable to report the temperature sensitivity in terms of the constant β.

Table 1. Oxygen permeability data.[a]

Membrane	Manufacturer and Description	Permeability[b] (P_i)	Reference
Polypropylene	Union Carbide (50% crystalline)	$3.7\times10^{-2}e^{-11400/RT}$	26
Polyethylene	Dupont (branched)	$8.9\times10^{-3}e^{-9900/RT}$	26
Polyethylene	Dupont (linear)	$2.3\times10^{-4}e^{-8800/RT}$	26
Polypropylene	Vorschein Folien-Fabrik[c] (W. Germany)	$1.7\times10^{-4}e^{-8090/RT}$	21
Teflon FEP	Dupont	$9.5\times10^{-6}e^{-5700/RT}$	29
Teflon TFE	Dilectrix	14.6×10^{-10} (38 °C)	4
Silicone rubber	Dow Corning		21
Water		$4.7\times10^{-11}e^{+3140/RT}$	30
Mylar	Mylar	5.78×10^{-11} (37 °C)	7

[a] Because of manufacturing variations, one should consult the manufacturer for accurate permeability data, if not using any of the sources listed.

[b] Expressed as $(cm^3\ (STP) \cdot cm)/cm^2 \cdot sec \cdot cm\ Hg)$.

[c] Marked with Radiometer P_{O_2} electrodes.

The influence of electrolyte layer thickness on the ratio of sensitivities at 25 and 38 °C is shown in figure 11. The inferred electrolyte thickness for polpropylene is 0.021

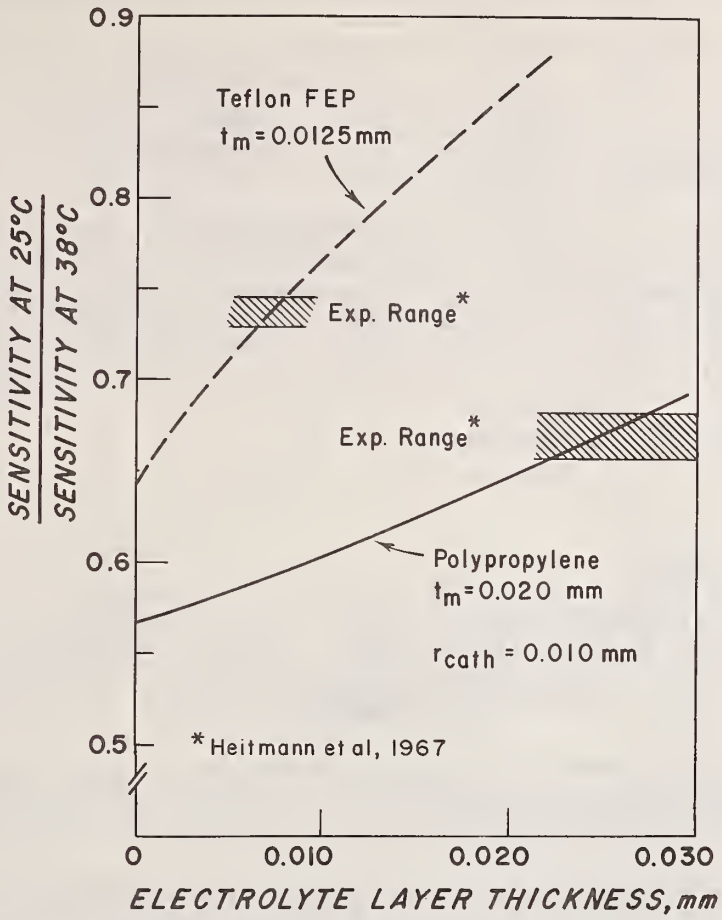

Figure 11. Influence of electrolyte layer thickness (ELT) on electrode temperature dependence. The experimental ranges indicated were obtained from Heitmann *et al.* [12]. The two curves were calcualted from eq. (12) using the data presented in table 1.

to 0.029 mm and that for Teflon FEP is 0.007 to 0.008 mm. These ranges agree remarkably well with those inferred on the basis of absolute electrode sensitivity.

Some authors have reported a much higher electrode temperature dependence, often approaching that of the membrane [22]. However, the cathodes used were large (macro-electrodes), in which case diffusion is mainly linear. In such cases, the effect of electrolyte temperature-dependence is not significant, but does not imply that the electrolyte layer is any thinner.

C. Influence of sample condition and characteristics on electrode sensitivity

As indicated by figures 6 and 7, the influence of sample stirring and the differences in electrode sensitivity between gas and liquid samples were predicted satisfactorily with only one value for electrolyte layer thickness. However, this is not a very sensitive test of the 3-zone model. Measurement of P_{O_2} in a stagnant liquid sample within a cuvette results in the greatest reduction of sensitivity of any sample media; let us examine this case further.

Variations in sample viscosity influence both the diffusion coefficient and the solubility coefficient of the sample [23]. By taking these influences into account, and a particular set of electrode conditions, one should be able to predict the influence of

sample viscosity on electrode sensitivity. Figure 12 presents the results of two such calculations, one for 0.00625 mm Teflon FEP and the other for 0.020 mm polypropylene.

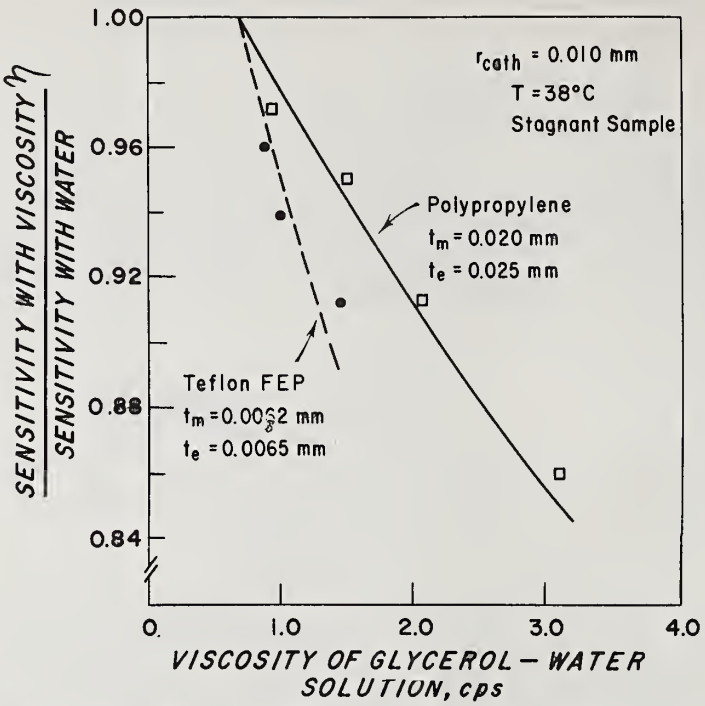

Figure 12. The influence of sample viscosity on electrode sensitivity. The experimental data •, □ taken from Heitman *et al.* [12], while the two curves (solid and dashed lines) were calculated from the data of table 1.

These two curves agree within 1 percent of the experimentally determined points. When the sample is whole blood, many other parameters besides viscosity must be considered (*e.g.*, variations in "permeability," convective settling, etc.). For this reason, calibration of the electrode with a solution of viscosity identical to the blood sample may reduce the discrepancy between blood and gas but not improve the consistency of the results. Rather, one must empirically determine, for a given electrode and cuvette, the solution whose viscosity results in the same relative reading as blood of a given hematocrit.

3. Discussion

We have presented a theoretical 3-zone model which predicts O_2 cathode behavior by taking into account membrane characteristics, electrolyte layer thickness, and finally, cathode diameter. Variation in sensitivity with cathode size has been reported to be nearly linear by Fatt [11], but this author assumed, on the basis of his model, that the findings represented anomalous electrode behavior. This is not at variance with our data (fig. 8). At a higher cathode size, the dependence does become of second order as predicted by linear diffusion models. As a matter of fact, any model that does not take into account the electrolyte layer thickness will predict a second order relationship between sensitivity and cathode size.

Staub [9] reported a microelectrode sensitivity of about 0.6×10^{-11} amp/mm Hg for a 0.050 mm diameter cathode, a value much lower than predicted by the 3-zone model for the thinnest available Teflon TFE membrane (0.00625 mm; sensitivity approximately 10×10^{-11} amp/mm Hg). This implies that when $r_c \gg t_m$ linear diffusion limits the electrode sensitivity.

The possible influence of other parameters on the behavior of microelectrodes does require consideration.

A. Membrane characteristics

The membrane serves two functions: (a) it is the medium in which the major oxygen gradient is established, and (b) it is the electrical isolator for the cathode. Polypropylene is the most commonly used membrane for static measurements. Others include Teflon[7] (both FEP and TFE), silicone rubber, and Mylar[7]. Ideally, the oxygen cathode-membrane system should exhibit no change in sensitivity with time in order to reduce the requirements for frequent calibration. Unfortunately, none of the membranes presently available fulfill these specifications, and we must consider their properties in more detail in order to anticipate their influence on cathode characteristics.

The above mentioned membranes are not truly homogeneous. Silicone rubber is always reinforced with silica particles (approximately 25 percent by weight). The other membranes have both crystalline and amorphous regions. Michaels and Bixler [24] have developed a model for permeation of gases through such membranes based on the concept that crystalline regions are impermeable to gases and act as an obstruction in an otherwise unmodified membrane. On this basis, permeability of the membrane can be expressed by the following equation:

$$P_m = \phi P_e^{-\Delta Ep/RT} \tag{15}$$

where $\phi P_e = \overline{P}$ from eq. (13).

The factor ϕ is generally a linear function of percent crystallinity. The latter depends on the manufacturing process, thermal treatment, and degree of stretching. Recent studies with polypropylene have shown a three-fold variation in permeability induced by mechanical stress [25]. These same studies indicated that stretched membranes will gradually "relax", resulting in a gradual increase in permeability subsequent to the initial stretching.

This phenomenon probably explains the cathode "aging" patterns which have been observed. The initial stretch which results when the membrane is first applied reduces permeability, but the subsequent stress-relaxation increases permeability, and therefore increases sensitivity with aging. However, there is an alternative explanation. Teflon-covered electrodes show a greater increase in sensitivity with "aging" than polypropylene [12]. Since stress-relaxation is accomplished by an extended deformation of the membrane, the increased sensitivity may be the result of a thicker electrolyte layer. To check this hypothesis, we examined the electrode temperature coefficient, β, on three succeeding days. As the sensitivity increased, β decreased, indicating a thicker electrolyte layer. Both explanations depend on the stress-relaxation phenomena, common to all the presently used membranes. At this time, there are insufficient data to decide between these two mechanisms.

The observation that polypropylene membranes produce electrolyte thicknesses of 0.020 to 0.030 mm has two important effects on electrode performance: (1) hydrodynamic drag can certainly be expected to effect the electrolyte thickness, and (2) the temperature sensitivity cannot be accurately predicted for a given electrode. Although this second fact is of great importance when one is considering the use of thermistors for automatic temperature compensation in oxygen electrodes, it is of little consequence in thermostated systems used routinely for static blood-gas measurements.

Table 1 presents the available permeability data for membranes commonly used with oxygen cathodes. The data from Buckles [21] were evaluated with polypropylene membranes available with one type cathode (Radiometer) and are distinctly different from the data of Myers, Stannett, and Szwarc [26]. This difference is undoubtedly due to variations in crystalline content [27].

Stiffness of the membrane determines its fit on the cathode surface. The flexible materials (*i.e.*, Teflon FEP) can be expected to conform closely to the shape of the cathode tip and to fold more smoothly over the outer cathode edges. The degree of stress-

[7] Trademarks of E. I. DuPont de Nemour Company.

relaxation, or "creep", is a function of the initial stretch; because of its lower "toughness", Teflon will exhibit the greater amount of stress-relaxation (for more detailed discussion of the mechanical properties of polymers, see ref. [3]).

B. Cathode geometry

Shape of the cathode tip has a pervasive influence on cathode sensitivity and stability. Since we are considering cathodes with diameters less than 0.1 mm, the radius of curvature is always much greater than the cathode radius. Yet in a previous section, we have shown that the surface need not be sharply pointed to result in a near-spherical diffusion pattern. Attempts at reducing the radius of curvature of the electrode tip to increase "spherical" diffusion is virtually impossible with microelectrodes. Therefore, even pointed electrodes are still "flat" in the region where diffusion occurs. A pointed electrode tip will result in an increased stress to the applied membrane; as a result, cathode instability is prominent.

C. Cuvette design

The microelectrode has been developed to eliminate the mechanical inconvenience of stirring and because it is desirable to use small blood samples without depleting its oxygen electrodes and their cuvettes have varied considerably and, as expected, the results have been inconsistent. According to Fatt [11], water flowing through a cuvette resulted in a signal deviation of 20 percent as compared with the stagnant sample. On the other hand, stirring did not result in any variation of the signal from that of the stagnant sample. Explanation for this disagreement was not given. If a sample flows past an electrode in one direction, hydrodynamic drag occurs in addition to surface renewal of sample. The hydrodynamic drag is not present when the sample is stirred symmetrically within the cuvette. Hydrodynamic drag is mainly dependent on the linear velocity across the membrane surface and sample viscosity (the linear velocity can be approximated by: electrode diameter x sample flow rate, ml/sec/cuvette volume in ml); it influences the electrode signal by modifying the membrane position relative to the cathode and by altering the electrolyte layer thickness.

The cuvette design also influences the long-term stability of the signal from a stagnant sample. Addition of a sample to the cuvette, previously equilibrated with a sample of lower P_{O_2} generally results in the current rising swiftly to a peak signal, then slowly dropping off. If the cuvette is a thin, dish-shaped space, the capillary forces will tend to hold the sample rigidly so that oxygen transfer from sample to membrane occurs only by diffusion. Thus, once the oxygen is consumed around the cathode, oxygen will have to diffuse farther to reach the cathode, resulting in a lower signal. In terms of the 3-zone model described above, one may say that

$$k = k_o e^{t/\gamma} \tag{16}$$

where t = time in seconds.

The time constant, γ, will depend on the cuvette design, being largest when the spacing is greatest. When the sample is not contained by capillary forces, natural convection will enhance simple diffusion of oxygen.

4. Appendix

A_i = area normal to flux, j_i, at a surface i (cm^2)

$C(r)$ = oxygen concentration, [ml (STP)/ml] at a radius r

D_i = oxygen diffusion coefficient of the material i (cm^2/sec)

F = Faraday units, (96,500 amp-sec/mol)

P_i = oxygen permeability of material i, expressed as

$$\frac{\text{ml (STP) - cm}}{\text{cm}^2\text{-sec-mm Hg}} \tag{17}$$

R = gas content (cal/g mol-K)

S^T = electrode sensitivity *i.e.*,

$$\frac{[\text{current for } P_{O_2} \text{ of ambient air}] - [\text{current when } P_{O_2} = 0]}{P_{O_2} \text{ (mm Hg) for ambient air}}$$

T = temperature (kelvin)

U_i and V_i = constants of integration

j_i = oxygen flux rate, ml (STP)/sec, across surface i

$$j_i = PA_i \frac{dP_{O_2}\text{-}l}{n_i} \tag{18}$$

k* = sample mass transfer coefficient, [ml (STP)/cm^2 - mm Hg - sec]

*The mass transfer coefficient to a spherical particle of radius r_m can be estimated as a function of the dimensionless velocity in a variety of ways [28]. However, at low Peclet Numbers (below 1.0 (see ref. [24]), the dimensionless transfer coefficient, r_m "k"/D, reaches a limiting value of 1.0. Thus,

$$k = D_m\alpha_m/r_m \times 760. \tag{19}$$

When stirring occurs, one generally finds that the ratio of the sensitivity with stirring to the sensitivity in a stagnant sample for an electrode immersed in electrolyte without a membrane, is 2:1 [12]. Thus, we may safely say that the k to be used when complete stirring occurs is $k = 2P_m/r_m \times 760$.

"k" = mass transfer coefficient to spherical particles, mol/mol sec

n = number of electrons that react to reduce one mol of O_2

n_i = vector normal to surface A_i

r = radius (cm)

$r_e = r_c + t_e$

$r_m = r_c + t_e + t_m$

t = thickness

α = Bunsen solubility coefficient [ml (STP)/ml-atm]

β = effective activation energy for oxygen diffusion in a micro-Clark-type oxygen electrode (cal/g mol)

ΔE_p = activation energy of permeation (cal/g mol)

γ = time constant for oxygen diffusion in a stagnant sample

ϕ = permeation factor; reflects dependence of permeation on crystalline content

Subscripts:

m = membrane
e = electrolyte
s = sample
c = cathode
O_2 = oxygen

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DYNAMIC RESPONSE OF A pCO_2 ELECTRODE

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1. Introduction

Gas sensing membrane electrodes are almost universally used for the measurement of the partial pressures of oxygen and carbon dioxide in blood. The basic operating principles have remained unchanged since the introduction of the PO_2 electrode by Clark [1][2] and the pCO_2 by Severinghaus and Bradley [2]. In each electrode the sample is separated from an electrolyte by a gas permeable membrane which allows transport of O_2 and/or CO_2. The diffusing gas is sensed in the electrolyte solution by: (a) reduction at a platinum or other noble metal cathode for O_2, or (b) measuring the change in pH in a buffer solution with a glass electrode for CO_2.

It is remarkable that despite the widespread use of these electrodes for nearly 20 years there have been few attempts to describe their response theoretically. Lucero [3] has considered some factors which affect the design of pO_2 electrodes from a practical standpoint. It was not until 1974 that an analysis was made of the oxygen profiles around a membrane covered pO_2 electrode [4]. Theory of the pCO_2 electrode was limited to the approximate equilibrium model given by Severinghaus [2] until a contemporary treatment by Ross *et al.* [5]. This model considered only the mass transport through the membrane. In the particular case of pCO_2 electrode, the kinetics of the reaction between CO_2 and water in the electrolyte solution can be rate limiting.

In this paper, a model for a pCO_2 electrode is proposed which considers mass transport in the membrane and in the electrolyte solution as well as chemical kinetics.

2. Mass Transport of CO_2

This model applies to a gas sensing electrode having the geometrical configuration shown in figure 1. The response of this electrode is determined by the concentration of gas (CO_2) as a function of time and distance from the ion detector. Once the concentration profile is established through both the membrane and the internal electrolyte phases, the CO_2 concentration may then be applied to the rate law governing the reaction:

$$CO_2(g) + H_2O(\ell) \rightleftarrows H_2CO_3(\ell) \overset{\text{fast}}{\underset{K_a}{\rightleftharpoons}} H^+ + HCO_3^-$$

The homogeneous reaction is assumed to be rate-limiting and even though the molecularity of reaction (1) is second order, the solvent mole fraction is very nearly one (*i.e.*, $C_{H_2O} >> C_{CO_2}$) resulting in an overall pseudo-first order reaction. The experimental basis for this assumption is the reported [6] decreased response time of the CO_2 gas-sensing electrode when carbonic anhydrase is incorporated in the internal electrolyte (*i.e.*,the enzyme catalyzes

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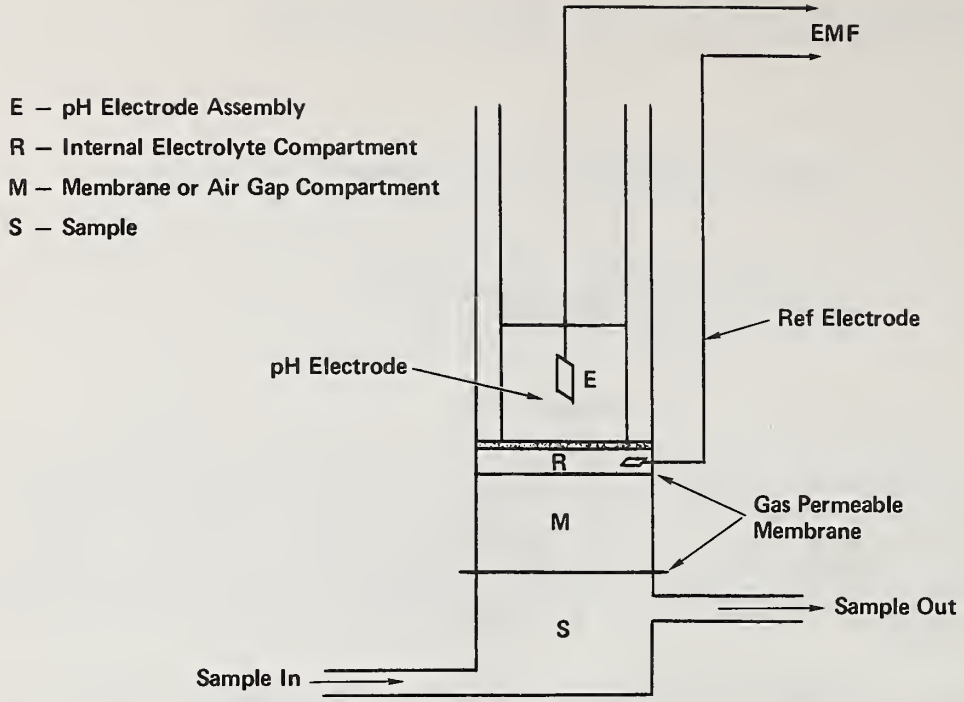


Figure 1. Geometric configuration of gas sensing electrode.

reaction (1)). Furthermore, the high sample concentration limit rarely exceeds 10^{-1} molar, representing a solvent to solute mole ratio of several orders of magnitude. The concentration of CO_2 in the sample is assumed to be invariant; this is equivalent to assuming that the sample is buffered in CO_2 and is stirred.

Consider the concentration gradient through the membrane and electrolyte media shown in figure 2. A steady state concentration of gas A (CO_2) is maintained in the sample compartment S. The membrane (M) in this case is simply an air gap of mostly nitrogen gas (B). The air gap may be maintained by a thin gas-permeable membrane delineating the sample/air gap interface (although the entire compartment M may be composed of a gas-permeable membrane, *e.g.*, silicone rubber). At the gas-liquid interphase S/M, which is denoted as X = 0, and through the membrane phase M bound at X = L, the gas phase concentration (C_A) is expressed in moles/liter or as a mole fraction N_A. N_A may, in turn, be defined as the partial pressure of A divided by the total pressure, P_A/P_{tot}, provided A and B form an ideal gas mixture. Gas B is inert to both the sample liquid and gas A and the partial pressures of both gases are in equilibrium with the liquid S.

At X = m corresponding to the gas-internal electrolyte interphase (M/R), the concentration is C_{A_m}. The entire system is controlled at constant temperature and pressure. The interface R/E at X = L separates the internal electrolyte from the pH glass electrode surface.

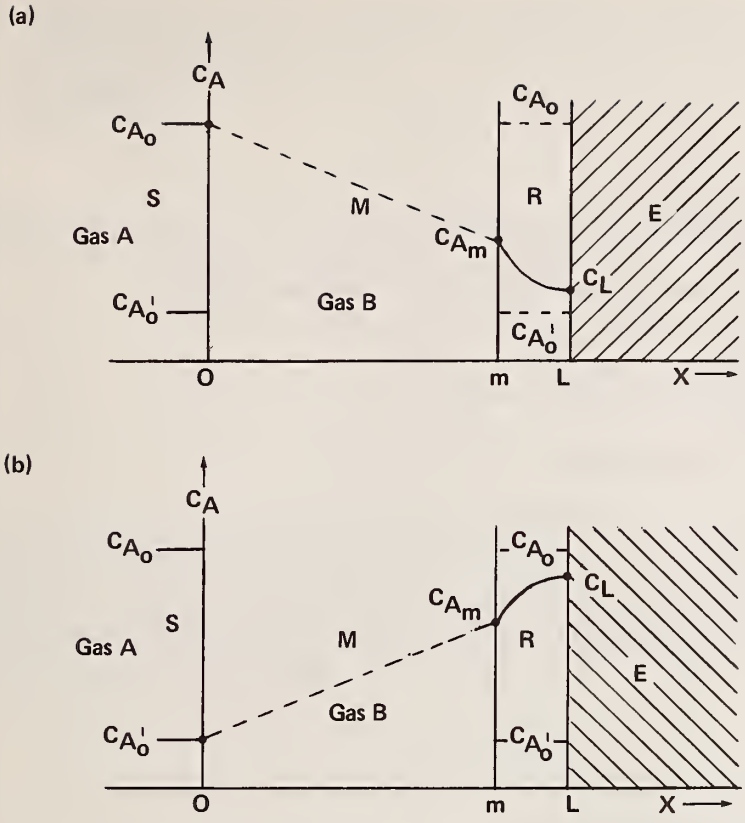
The boundary conditions for this mass transport problem are:

$$X = 0 \qquad C_A = C_{A_o}$$

$$X = m \qquad C_A = C_{A_m}$$

$$X = L \qquad \left(\frac{\partial C_A}{\partial x}\right)_{X=L} = 0$$

The condition at X = L states that no H_3O^+ diffuses through the pH glass membrane. This is



(a) Low-to-high concentration change

(b) High-to-low concentration change

S — Sample

M — Membrane and Air Gap

R — Internal Electrolyte

E — pH sensing Electrode

Figure 2. Concentration profiles at electrode-sample interface.

a reasonable assumption since the diffusion coefficient of H_3O^+ through glass is much smaller than the diffusion coefficients of A in B (D_{AB}) and A in R (D_{AL}).

The electrode is subjected to an instantaneous step concentration change from C_{A_o}' to C_{A_o}. The resulting concentration gradient is the driving force for molecular diffusion of A into M and subsequently into R. Solving the mass balance equation through region M, one obtains the following equations:

$$C_A = \left(C_{A_m} - C_{A_o}\right) \frac{x}{m} + C_{A_o} \qquad (1)$$

or

$$\frac{\left(P_{tot} - P_A\right)}{\left(P_{tot} - P_{A_m}\right)} = \left(\frac{P_{tot} - P_{A_o}}{P_{tot} - P_{A_m}}\right)^{x/m} .$$

The flux at the gas-liquid interface M/R is:

$$\left(J_A\right)_{x=m} = -\frac{D_{AB}}{m}\left(C_{A_m} - C_{A_o}\right) = \frac{P_{tot}\, D_{AB}/RT}{m} \ln\left(\frac{1 - P_{A_m}}{1 - P_{A_o}}\right) \quad . \qquad (2)$$

As gas A diffuses through medium R, it is chemically transformed to compound Q (H_2CO_3) by

reaction (1). The total mass transport equation including homogeneous chemical kinetics is:

$$- D_{AL} \frac{\partial^2 C_A}{\partial x^2} + (k_1 + k_2)\, C_A - k_2 \left(C_{A_o} - C_{Q_o} \right) = 0 \qquad (3)$$

where C_{A_o} and C_{Q_o} are the initial concentrations of A and Q. The solution to the above equation for the above boundary conditions defines the concentration profile through region R:

$$C_A = \frac{C_{A_m} - \beta}{\gamma} \left[e^{\alpha^{1/2} x} + e^{\alpha^{1/2} (2L - x)} \right] + \beta \qquad (4)$$

where

$$\alpha = \frac{k_1 + k_2}{D_{AL}}, \quad \beta = \frac{k_2}{k_1 + k_2} \left(C_{A_o} - C_{Q_o} \right)$$

and

$$\gamma = e^{\alpha^{1/2} m} + e^{\alpha^{1/2} (2L - m)}$$

The flux at X = m is:

$$(J_A)_m = - D_{AL} \alpha^{1/2} \left(C_{A_m} - \beta \right) . \qquad (5)$$

From eqs. (2) and (5) an expression for C_{A_m} is derived:

$$C_{A_m} = \frac{m \left(\frac{D_{AB}}{D_{AL}} \right) \beta \left[\alpha^{1/2} \zeta - 1 \right] - C_{A_o}}{m \left(\frac{D_{AB}}{D_{AL}} \right) \alpha^{1/2} \zeta - 1}$$

where

$$\zeta = \gamma^{-1} \left[e^{\alpha^{1/2} m} - e^{\alpha^{1/2} (2L - m)} \right] . \qquad (6)$$

The removal of geometrical dependence on the ion sensing element in the internal electrolyte can be advantageous for practical applications. This could be accomplished by

designing a sensor so as to make it responsive to an average ion concentration as, for example, a cylindrical pH sensor enveloping the internal electrolyte compartment. The concentration of A may be averaged throughout the entire volume of compartment R resulting in the following relationship:

$$C_{avg.} = \int_m^L C_A dx \Big/ \int_m^L dx$$

$$C_{avg.} = \frac{\left(C_{A_m} - \beta\right)\zeta}{(L - m)\alpha^{\frac{1}{2}}} + \frac{\beta}{(L - m)} \quad . \tag{7}$$

3. Time Response

Equations (1), (2), (4), and (5) accurately describe within the confines of the model the steady state concentration of CO_2 (gas A) and the rate of mass transport. However, no information about the response time of the electrode can be derived from the above treatment thus far. Since the CO_2 gas sensing electrode is slow responding due to a rate limiting chemical reaction, eq. (4) may be applied for the appropriate distance X (at X = L in this case) to the rate law for reaction (1). An expression for the time response is obtained by solving the first order rate equation for reaction (1):

$$-\frac{dC_A}{dt} = (k_1 + k_2)\, C_A - k_2 \left(C_{A_o} - C_{Q_o}\right) . \tag{8}$$

The solution of eq. (8) yields an expression for the time required to reach the equilibrium concentration C_{A_e} at distance X = L:

$$\ln \left[\frac{C_{A_o} - C_{A_e}}{C_A^L - C_{A_e}} \right] = (k_1 + k_2)t \quad .$$

The following mass action law can then be applied:

$$H^+ = \frac{K_a\, k_1 [CO_2]}{k_2 [HCO_3^-]} \tag{9}$$

to describe the time to reach a given pH change as:

$$t = (k_1 + k_2)^{-1} \ln \left\{ \frac{C_B\left(C_{H_o^+} - C_{H_e^+}\right) + \left(C_{H_o^+}\, C_{(HCO_3^-)} - C_{H_e^+}\, C_{(HCO_3^-)_e}\right)}{C_B\left(C_{H^+} - C_{H_e^+}\right) + \left(C_{H_o^+}\, C_{(HCO_3^-)_o} - C_{H_e^+}\, C_{(HCO_3^-)_e}\right)} \right\} \tag{10}$$

where C_B is the concentration of internal electrolyte (usually $NaHCO_3$) and $C_{HCO_3^-}$ is that

formed from CO_2. Equation (10) reveals that the time response is inversely proportional to the rate constants and logarithmically dependent on ion concentration changes although in a complex manner. In the high concentration limit of detection, the square terms become insignificant reducing the logarithmic term to the simple ratio of $\left(C_{H_o^+} - C_{H_e^+}\right) \div \left(C_{H^+} - C_{H_e^+}\right)$.

The time required to reach equilibrium via steady state mass transport may also be determined. The flux at, *i.e.*, X = m may be equated to $d\left(C_{A_m}\right)/dt$;

$$- \frac{dC_{A_m}}{dt} = - D_{AL}\, \alpha^{\frac{1}{2}} \left(C_{A_m} - \beta\right) \quad .$$

Integration of the above equation yields the following expression for time:

$$t = \left[D_{AL}(k_1 + k_2)\right]^{-\frac{1}{2}} \ln \left\{ \frac{C_{A_m} - \beta}{\xi_o C_{A_m}} \right\} \tag{11}$$

where ξ is the reduced concentration parameter $\left(C_{A_m} - \beta\right)/\beta$ and ξ_o is defined as the fractional reduced concentration. In terms of the average concentration change in compartment R:

$$t = \left[D_{AL}(k_1 + k_2)\right]^{-\frac{1}{2}} \left(\ln \left\{ \left[(L - m)\, C_{avg} - \beta\right] \frac{\alpha^{\frac{1}{2}}}{\xi_o} \right\} - \ln \left\{ \left[(L - m)\, C_{avg} - \beta\right] \alpha^{\frac{1}{2}} + \xi_o \beta \right\} \right) . \tag{12}$$

The reverse process considering a high to low step concentration change is similarly treated, resulting in virtually identical relationships with the exception that $\beta' = [k_1/(k_1 + k_2)]\left(C_{Q_o} - C_{A_o}\right)$ substitutes the expression for β.

4. Discussion

The sensitivity and detection limit of a CO_2 gas sensing electrode are determined by the equilibrium reaction (1) and by eq. (9). The detection limit is also a function of the resolution of the pH electrode; for a limit of 0.01 pH unit the CO_2 concentration detection limit can be calculated from eq. (9) to be approximately 10^{-5} *M*. The equilibrium response of the electrode is not of prime consideration here, but rather the time-dependent response to changes in CO_2 concentration.

Equations (1) and (4) give the steady state CO_2 concentration profiles through regions M and R following a step change in sample concentration. Figures 3 and 4 show numerical solutions to these equations under different conditions. In figure 3, conditions correspond to a near ideal CO_2 sensing electrode. Diffusion of CO_2 across the membrane region M is almost unimpeded, *i.e.*, the membrane is an "air gap." However, it must be noted that diffusion of CO_2 into the electrolyte region R is of much greater importance, even though the thickness of layer R is much less than M. The curve shown in figure 4 corresponds to a more general case in which layer M represents a diffusion barrier, *i.e.*, it is a gas-permeable plastic membrane.

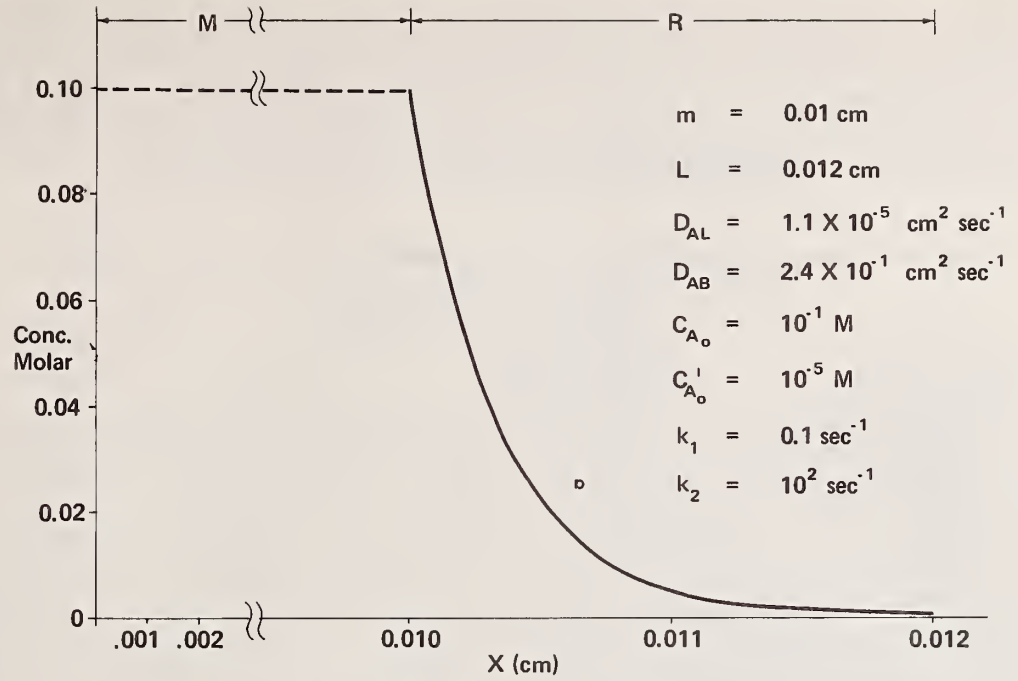


Figure 3. Concentration profile for CO_2 electrode where the membrane M is an air gap.

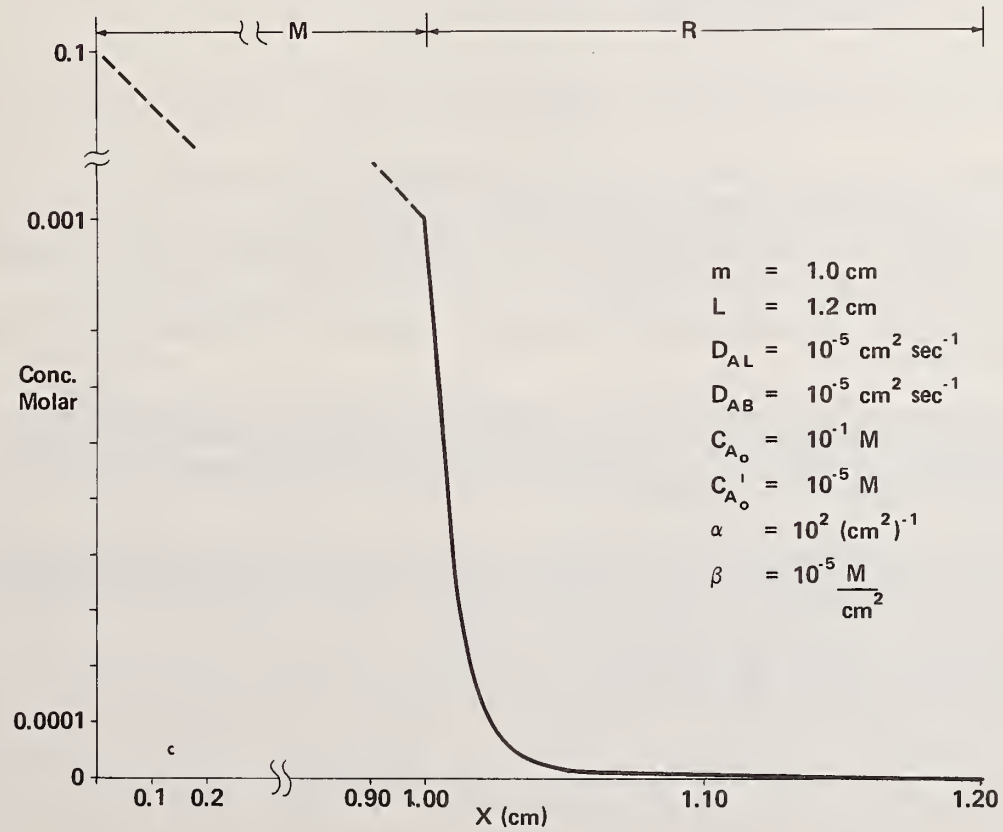


Figure 4. Concentration profile for a generalized gas sensing CO_2 electrode where the membrane M is a gas permeable plastic.

The time response of the electrode is given by eq. (10). Table 1 shows some calculated values for 99 percent response times for various concentration changes. It must be noted that the time response for a high-low concentration change is greater than for the reverse. The magnitude of the differences in these response times is a function of the homogeneous chemical kinetic rate constants and the diffusion coefficient of CO_2 in the electrolyte region R. In the case of the diffusion rate limited process, the thickness of regions R and M effect the response time equation.

Table 1. Time required to achieve 99 percent of concentration change .

Initial concentration (moles/liter)	Final concentration (moles/liter)	$t\left[D_{AL}(k_1 + k_2)\right]^{1/2}$ eq. (11) / $t\,(k_1 + k_2)$ eq. (10)
10^{-5}	10^{-3}	4.61
10^{-5}	10^{-2}	4.61
10^{-5}	10^{-1}	4.61
10^{-1}	10^{-3}	9.21
10^{-1}	10^{-4}	11.51
10^{-1}	10^{-5}	13.82

The results obtained using this model of a CO_2 sensing electrode agree in principle with the known experimentally determined properties. It is hoped that the model will lead to a clearer understanding of those factors which contribute to the electrode response and to improvements in electrode performance.

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MONITORING OF OXYGEN PRESSURE IN HUMAN AND ANIMAL BLOOD

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The surveillance of arterial oxygen pressure (PaO_2) in patients with pulmonary or cardiac failure by frequent sampling of blood has several obvious disadvantages. Continuous monitoring of PaO_2, including recording of variations in PaO_2 with respiration and cardiac activity, provides more useful information, but this sophisticated monitoring by use of catheter electrodes is still affected by numerous problems. The size of the sensor as well as stability, flow dependency and transient response are crucial factors. Low flow dependency and fast response oppose each other [1][1], but these requirements calling for an optimal compromise in any individual situation have received relatively little attention since a fast response has often been considered to be unimportant, particularly in clinical monitoring. It cannot be ignored, however, that a fast response is often necessary or at least desirable and may provide interesting accessory information [2-4]. We have been paying particular attention to this aspect for a number of years.

1. General Problems and Requirements of PO_2 Electrodes in Continuous Monitoring

The problems and requirements of PO_2 electrodes in continuous monitoring *in vivo* include technical as well as biological aspects. For the evaluation and appraisal of any such system the following check list may be useful:

A. Sensitivity and accuracy including linearity, reproducibility, short-term stability, long-term stability, shelf life, transient response, zero current (value and drift), flow dependency, temperature dependency, sensitivity to CO_2, sensitivity to other gases, sensitivity to pressure, to acceleration, and to water vapor (water deposition).

B. Design and constructive lay-out including miniaturization, safety, and complexity of servicing and operation.

C. Sterilization.

D. *In vivo* parameters including biocompatibility (toxicity, blood changes, tissue reactions, blood coagulation, etc.), mechanical and electrical compatibility (flow distortion, tissue changes, electroshock, membrane potential changes), influences of the medium on the sensor (protein deposition, blood coagulation at electrode, mechanical stress, galvanic reactions).

Intensive research during the past years has led to the solution of most problems connected with the technical parameters sensitivity, accuracy, constructive lay-out, and sterilization. For human applications, however, improvement of the constructive lay-out remains desirable, particularly with respect to the size of the transducer. For prolonged monitoring of human PaO_2 the main issue, however, is the solution of the problems connected with the *in vivo* parameters, particularly the biocompatibility of the catheter electrode and the influences of the medium on the sensor. But also the mechanical compatibility and the positioning of the transducer are important in certain circumstances. Biocompatibility of a catheter electrode is never complete but can be improved by a careful construction of the sensor and by use of proper materials including coating the tip of the catheter with silicone. Protein and blood cell deposition as well as blood coagulation at the tip of the

[1]Figures in brackets indicate the literature references at the end of this paper.

electrode affect fast electrodes much more than slow ones. Since these phenomena also involve a certain risk for the patient, it is advantageous that their effects on the fast electrodes become noticeable quite readily and thus provide a warning at an early stage.

Trials of monitoring PaO_2 in the human radial artery using our ring electrode [5] revealed the necessity of a catheter probe with a diameter smaller than 2 mm. First, this problem was circumvented by measuring PaO_2 in an arterio-venous shunt (as described below), but simultaneously a new electrode with a diameter of 1.2 mm and essentially similar characteristics as previous designs has been developed. These two lines of approach will now be discussed.

2. Monitoring of Human PaO_2

Jank, *et al.* [6] applied our previously developed type of ring electrode [5] to patients by inserting it into a specially made shunt between the radial artery and the antecubital vein. A slight disadvantage of this system is a possible reduction of the peripheral circulation due to the shunt. On the other hand, this system offers several important advantages. The markedly increased blood flow in the shunt (as compared with normal radial arterial flow) decreases the risk of blood coagulation (which is further minimized by coating with silicone and continuous instillation of heparin through the arterial end of the shunt), provides thermostating of the metal block holding the electrode by the blood itself, and secures a well-defined flow situation which excludes artifacts due to the remaining flow sensitivity of the electrode. Further advantages of this shunt arrangement are that calibration can be checked at every desired moment, the transducer can be replaced at any moment if necessary, and other parameters may be measured simultaneously. Cathode and Teflon membrane are sterilized at 55 °C with ethylene oxide, the Silastic shunt tube by germicide. The system, as tested *in vitro*, is stable (variation less than 0.5 percent in 24 hours), linear and precise (± 0.2 percent) in a broad range of oxygen pressures (from about 10 to more than 700 mm Hg); its response time for 95 percent deflection is 0.4 second.

Continuous recording of PaO_2 was achieved for periods of 6 to 24 hours in more than 100 patients, mainly with respiratory failure. The PO_2 of blood samples taken from the shunt of each patient was compared with the continuous PaO_2 readings. The values tallied well up to 110 mm Hg. A systematically lower PO_2 measured in the sample from 110 mm Hg upwards is likely due to leakage from the syringe and oxygen consumption by the erythrocytes during transport and analysis of the blood samples. This system may be reliably used for monitoring PaO_2 in patients up to 24 hours without occurrence of any cutaneous or vascular reactions.

3. New Miniature PO_2 Electrode

Our new electrode has a lay-out similar to our previous design [5] and also a response time for 95 percent deflection of 0.4 second, but a diameter decreased from 2 to 1.2 mm [7,8]. The cathode again is a platinum ring of 0.3 mm diameter and approximately 3 μm thickness which makes possible a very low flow dependency in the presence of a relatively high current output. In order to guarantee a fast sufficient electrolyte exchange in the space between the membrane and the cathode area (which is important for the stability and small CO_2 sensitivity of the electrode) the ratio of the unsupported to total membrane area had to be increased from 4 to 6 percent. Since this might lead to an increased sensitivity to pressure, the static and dynamic sensitivity to pressure was investigated with particular care. The most important characteristics of the probe are (with a 6 μm Teflon membrane at 37 °C):

sensitivity = about 0.6 nA/mm Hg,
linearity = ± 0.2% (from 10 to 760 mm Hg),
reproducibility = better than 0.1 mm Hg,
short-term stability (2 h) = ± 0.25%,
long-term stability (24 h) = ± 1%,
response time for 95% deflection = 0.4 s,
zero current ≲ 15 mm Hg (drift 0.5 mm Hg/24 h),
flow dependency (7-100 cm/s) = ± 1%,
temperature dependency (nonlinear) = 2-3%/1 °C,
sensitivity to physiological CO_2 ≲ 2 mm Hg,
sensitivity to pressure: static ≲ 0.2%,
dynamic ≲ 1%,
sensitivity to acceleration (0 to 10 g) ≲ 0.1%.

This electrode so far has been applied to physiological studies in the dog and cat as well as to continuous monitoring of human PaO_2 in the radial artery where it was introduced through a Teflon needle. Figure 1 shows a tracing in a cat. In the animal experiments where the catheter tip was protected by silicone oil, no blood coagulation occurred at the tip for several hours.

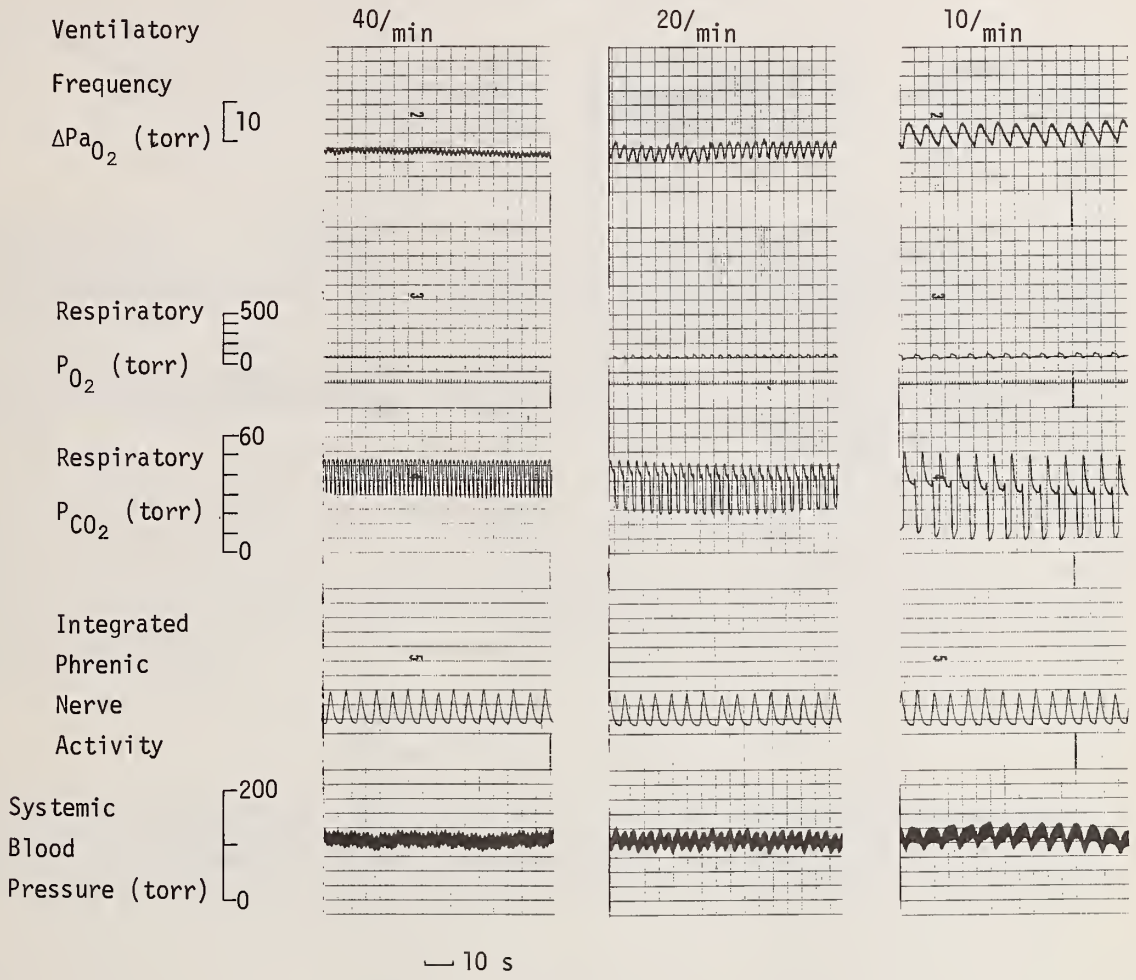

Figure 1. Recording of arterial oxygen pressure (PaO_2; top tracing) in the carotid artery of an artificially ventilated cat during an experiment concerning the regulation of breathing. The respiratory frequency was changed from 40/min to 10/min, while end-expiratory PO_2 and PCO_2 were kept constant by adjusting the inspiratory concentrations. Note the respiratory fluctuations of PaO_2 (ΔPaO_2), increasing with decreasing respiratory frequency, which can be followed faithfully by these fast PO_2 electrodes (for proof, see reference 4).

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ALTERNATIVE METHODS OF CO_2 MEASUREMENT, WITH PARTICULAR REFERENCE TO CONTINUOUS RECORDING

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Continuous *in vivo* recording of CO_2 pressure (PCO_2) in the gas phase has long been common but continuous *in vivo* monitoring in fluids, particularly blood, has been impeded by the relatively large dimensions and the slow response of the available devices. A method of measuring PCO_2 in liquids accurately and rapidly by a miniaturized instrument analogous to the oxygen electrodes developed by Kreuzer and his coworkers [1-3][1] presumably might help to solve many problems in physiology and reaction kinetics, as well as in clinical medicine.

The conventional CO_2 electrode according to Stow *et al.* [4,5] has been widely used but remains limited to a response time of the order of one minute in most cases, particularly with miniaturized types which furthermore are apt to be less stable and have a shorter life time. The recently introduced General Electric catheter CO_2 electrode [6], *e.g.*, still has a response time of one minute. A recent calorimetric CO_2 electrode [7] also has a response time of 2 minutes for 99 percent deflection. We therefore had decided to investigate the possibility of applying other principles to the measurement of CO_2. In the present paper, we will discuss the results of our experience with the quinhydrone electrode and with the conductivity electrode.

1. The Quinhydrone Electrode

The quinhydrone system was previously applied to pH measurement and has now been modified for the determination of PCO_2 [8]. The principle resides on the oxidation and reduction of hydroquinone and quinone, respectively; the oxidation-reduction reaction and the corresponding electrode potential are described by:

$$Q + 2H^+ + 2e^- \rightleftharpoons H_2Q \tag{1}$$

$$E = E_o + \frac{RT}{2F} \ln \frac{a_Q (a_{H^+})^2}{a_{H_2Q}} = E_o + \frac{RT}{2F} \ln \frac{a_Q}{a_{H_2Q}} + \frac{RT}{F} \ln a_{H^+} \tag{2}$$

where Q = quinone; a_Q, a_{H_2Q}, a_{H^+} = ionic activities of quinone, hydroquinone and hydrogen ions, respectively; E = electrode potential; E_o = standard electrode potential = 700 mV at 25 °C; other symbols as usual.

If the activities of quinone and hydroquinone are the same, the electrode potential becomes independent of any of these species and is a function of the hydrogen ion activity only. This condition prevails when using quinhydrone, an equimolar compound of quinone and hydroquinone.

[1]Figures in brackets indicate the literature references at the end of this paper.

A membrane-covered electrode was constructed with either a calomel or a silver-silver chloride electrode as a reference, either of which is suited. Figure 1 shows the construction of this electrode. A platinum ring (o.d. 4.9, i.d. 4.5 mm) was mounted around the liquid junction of a calomel electrode, or a platinum plate (surface area 8 mm^2) together with a silver ring (o.d. 6.8, i.d. 5.8 mm) was melted into glass (not shown here). A membrane (Silastic 25 μm or Teflon 6 μm) was mounted and the electrode housing was filled with a solution of 2 ml quinhydrone (10^{-3} mol/l) and KCl (0.1 mol/l) with or without sodium bicarbonate (10^{-3} mol/l). Lens paper was used as a spacer to stabilize the fluid layer.

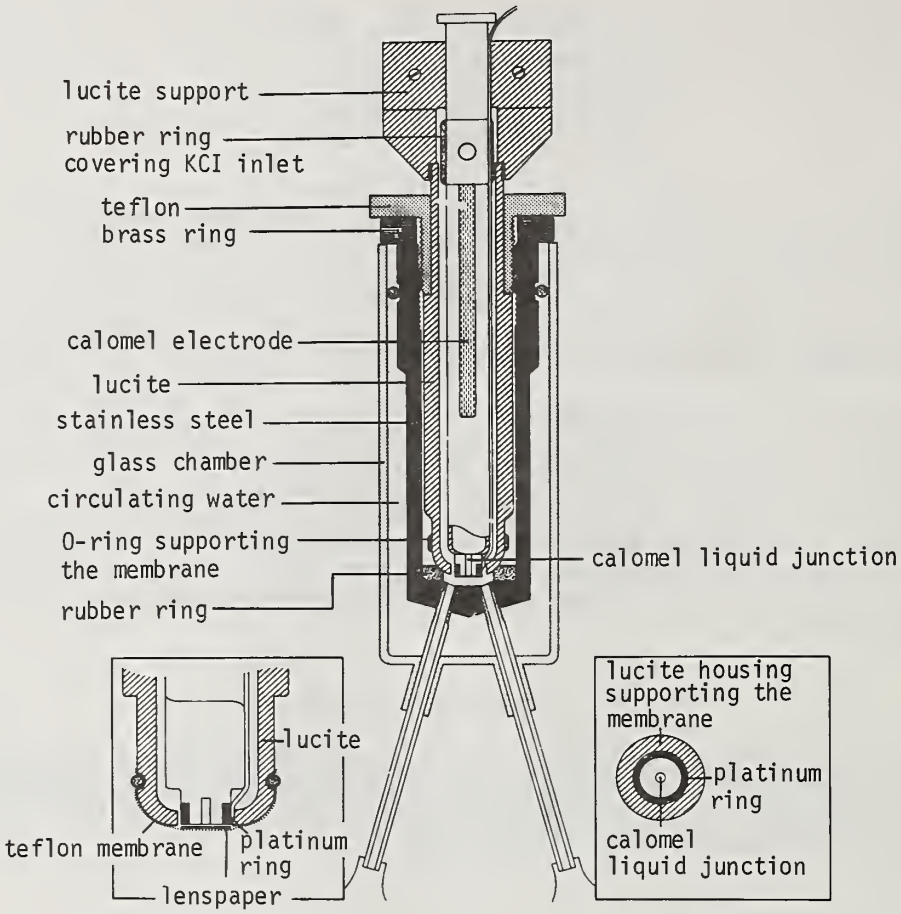

Figure 1. Construction of CO_2-quinhydrone electrode [8] (with permission of *Respir. Physiol.*).

The pK of hydroquinone is 10.32 ± 0.08. The plot of E *vs* pH is linear between pH 4 and 9, with a slope $-\Delta E/\Delta pH$ = 58.2 mV/pH at 25 °C, the same with or without CO_2. The plot of E *vs* log PCO_2 with 0.1 mol/l KCl is linear between PCO_2 15 and 80 mm Hg, with a slope $-\Delta E/\Delta \log PCO_2$ = 55.8 mV/log PCO_2 at 25 °C in the presence of bicarbonate. This results in a $-\Delta pH/\Delta \log PCO_2$ = 0.96, in good agreement with the conventional CO_2 electrode.

A number of factors such as chloride concentration, oxygen concentration, light, time, acid-base reactions of hydroquinone and quinone, semiquinones, autoxidation, and salts influence the performance of this electrode, but it could be shown that their effect in 0.1 mol/l KCl is negligible in physiological conditions [9].

The most important characteristics of performance of this electrode, to be compared with the conventional CO_2 electrode, are listed in table 1. Calibration lines are shown in figure 2 in bovine blood at 37 °C with or without bicarbonate added to the electrode solution.

Table 1. Synopsis of properties of various CO_2 electrodes for continuous monitoring *in vivo*.

Property	Stow electrode	General Electric catheter probe (Stow principle)	Quinhydrone electrode macro	Quinhydrone electrode micro	Conductivity electrode	Calorimetric electrode
Accuracy	2%	±2-3 mm Hg	similar to Stow electrode		<2 mm Hg	SE = ±5 mm Hg
Calibration	linear	linear	linear	linear	linear	linear
Response time	1 min for 99.5%	67 s for 90% (*in vivo*)	2 min for 95% on 3 min for 95% off	1 min for 95%	4-7 s for 90%	2 min for 99%
Stability	0.2 mV/h	±3 mm Hg during 6 h	<0.2 mV/h	4 mV/h (platinized)	?	±5 mm Hg during 1 week

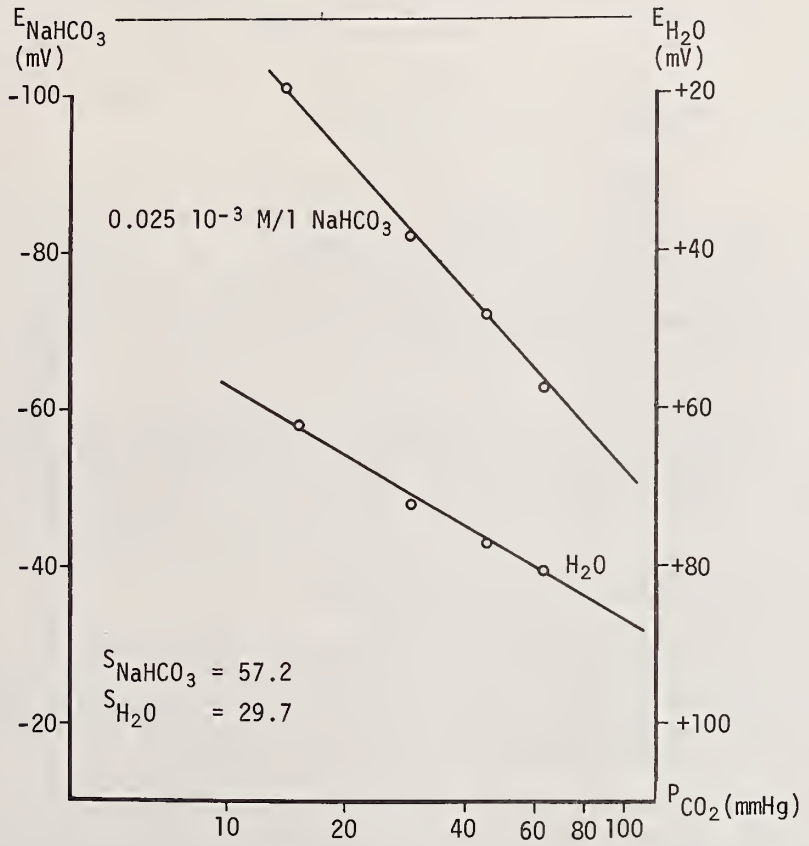

Figure 2. Calibration lines of CO_2-quinhydrone electrode with bovine blood equilibrated with various CO_2 concentrations at 37 °C with or without bicarbonate added to electrode solution [8] (with permission of *Respir. Physiol.*).

The performance of this electrode is fully comparable to that of the conventional CO_2 electrode. Compared with the latter, it has the advantage of being an inexpensive low-impedance electrode which may be miniaturized more easily and therefore might be adapted to *in vivo* PCO_2 estimation.

2. A Single-Unit Electrode for CO_2 and O_2

We also miniaturized the CO_2-quinhydrone electrode and combined it with a polarographic oxygen electrode of the Clark-type, yielding a single-unit CO_2-O_2 sensing microelectrode system. The design of this electrode is similar to that of the oxygen electrode constructed by Schuler and Kreuzer [1] and Kimmich and Kreuzer [2]; a slightly larger version is shown in figure 3 [10].

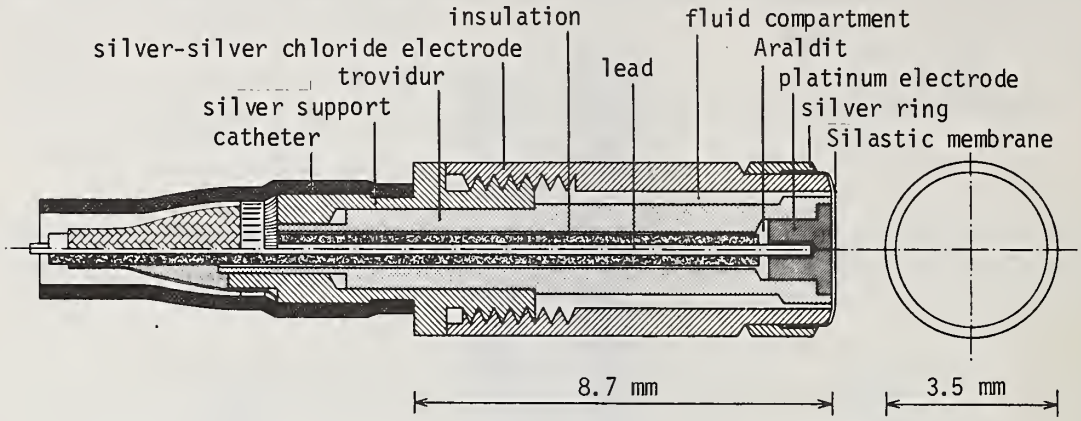

Figure 3. Construction of CO_2-quinhydrone microelectrode system [10] (with permission of *Respir. Physiol.*).

The cylindrical probe has an external diameter of 3.5 mm and a length of 8.7 mm. It consists of a central platinum electrode with a surface area of about 2.5 mm^2 sealed with Araldite into a holder of polyvinyl chloride (Trovidur) and surrounded by a tubular Ag-AgCl reference electrode. The tip of the probe is covered by a thin membrane (Silastic 25 µm or Teflon 6 µm) fixed to the reference electrode by a silver ring. A quinhydrone solution of 10^{-3} mol/l in 0.1 mol/l KCl with or without bicarbonate added, is introduced into the probe.

The performance data of this probe used as a CO_2 electrode are listed in table 1. Figure 4 shows linear calibration lines for blank and platinized platinum electrodes. The line of the blank probe lies somewhat above that of the platinized probe. The slopes with 0.1 mol/l KCl are similar and lower than in the macroelectrode described above. The sensitivity $-\Delta pH/\Delta \log PCO_2$ thus is about 0.42. Stability is better in the platinized probe but considerably inferior to that of the macroelectrode described above. Response time, however, is superior to that of the macroelectrode.

The properties of this probe used as an oxygen electrode are fully comparable with those of the oxygen sensors described previously by Kreuzer and his group, particularly also concerning the fast response, the response time for 95 percent deflection being about 0.4 second at 20 °C, independent of the electrolyte composition. The polarogram is not affected by addition of quinhydrone with or without bicarbonate.

The responses of this unit used as a CO_2 electrode to oxygen and used as an oxygen electrode to CO_2 are mutually independent in physiological conditions, at least as long as the succession is not too fast. In view of a response time for 95 percent deflection of about one minute for CO_2 and about 0.4 second for oxygen, it is possible to take successive recordings of oxygen and CO_2 *in vivo* after elapse of the respective response times.

There is a lower limit for the surface area of the electrode concerning CO_2, being reached in the blank platinum electrode at a surface area of about 1 mm^2, which is important for further miniaturization. The platinum surface of this electrode is too large for the determination of oxygen in fluids since the flow dependency is considerable. Any compromise in this respect may be difficult without membranes combining high permeation rate for CO_2 (in order to get a sufficiently fast response) with poor permeability to oxygen.

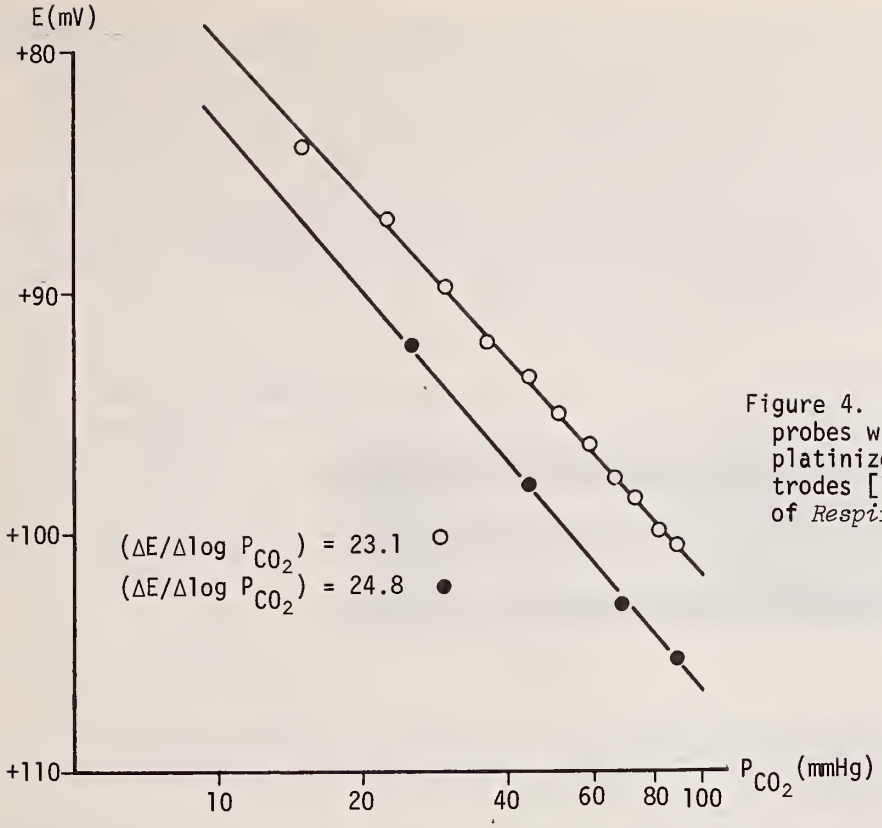

Figure 4. E *vs* PCO_2 of CO_2 probes with blank (o) and platinized (•) platinum electrodes [10] (with permission of *Respir. Physiol.*).

3. The Conductivity Electrode

In a further attempt to reduce the response time which is still too long in the types of quinhydrone electrode just described, an entirely different principle was adopted in the device to be presented now, in which the principle of establishing a CO_2 equilibrium across the electrode membrane has been abandoned. Electrolyte conductivity has proven to be a fast, sensitive and accurate method for the determination of CO_2 in liquids. The microelectrode system presented here is a first attempt to apply conductometry to continuous monitoring of PCO_2 *in vivo* [11].

A stainless steel tip is attached to one end of an x-ray double-lumen polyethylene catheter of 60 cm length, 2.7 mm external diameter and 0.8 mm lumen diameter. A Silastic membrane of 25 μm is mounted on this tip by a device specially designed for this purpose and fixed by a stainless steel ring. Each lumen is separately connected to a conductivity cell located at some distance from the membrane. The whole electrode system is flushed continuously with bidistilled water at a constant flow rate. Electrolyte conductivity of the water is measured before entering the catheter and after having passed the membrane. The difference in conductivity between inflowing and outflowing water is related to the PCO_2 of the medium to which the electrode is exposed.

The sensitivity of the electrode system, which is the change in specific conductivity (in $S \cdot cm^{-1}$) of the carrier to a change in PCO_2 of the medium, is determined, among other factors, by the efficiency of CO_2 transfer from the medium across the membrane to the carrier. It depends on membrane permeability and surface area, contact time of the carrier with the membrane (contact length/flow velocity), and carrier volume per unit membrane surface area (carrier layer thickness). In this electrode prototype, a membrane surface area of 3.5 mm^2 and a layer thickness of 250 μm were chosen. Carrier flow rate was within a range of 1 to 7 ml/min, thus giving contact times between 0.05 and 0.007 second.

The choice of the conductivity cell depends on the specific conductivity to be measured. Since at a specific conductivity of the order of $\mu S \cdot cm^{-1}$, variations of a few percent have

to be determined, a conductivity cell with a low cell constant (= distance between the two plates/surface area of one cell plate, in cm^{-1}) is required. The cell volume should be close to that of the carrier in contact with the membrane in order to maintain the upper limit of the frequency at which PCO_2 fluctuations of the medium can be followed by the electrode. A series of 6 types of cell were investigated from which the type shown in figure 5 was most suited. It consists of two platinum wires (0.2 x 0.05 mm) coated by Araldite and wound at a distance of 0.2 mm to a coil of 0.6 mm i.d. and a length of 8 mm (the interior of the coil being stripped of insulating Araldite), fitting into the lumen of the catheter tip. The main advantage of this location is the reduction of the response time and the convenience of temperature control. Between uses, the cells were filled with Hibitane 0.2 percent to prevent microbial contamination.

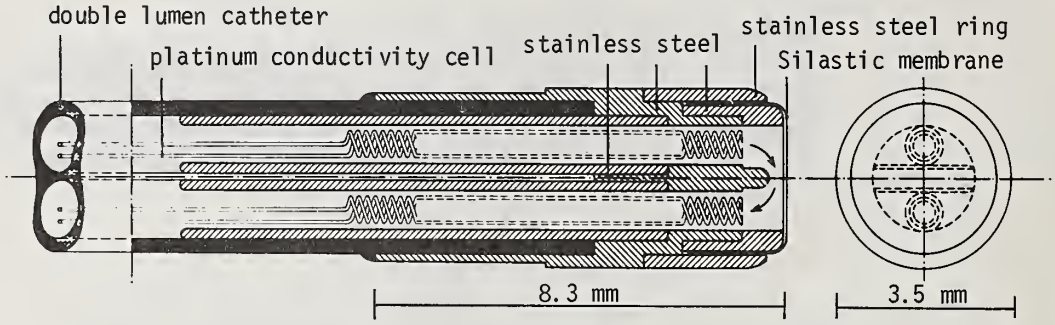

Figure 5. Stainless steel conductivity electrode tip covered by a CO_2-permeable membrane provided with a spiral conductivity cell and mounted on a flexible catheter [11] (with permission of *Respir. Physiol.*).

Electrolyte conductivity was determined with a commercially available direct-reading conductivity meter (Radiometer CDM 2, frequency 70 Hz, test voltage 0.25 V) connected to a two-channel recorder. Conductivity meter and recorder were calibrated by precision resistance boxes. In this way, it was possible to estimate variations in specific conductivity of up to 0.02 $\mu S \cdot cm^{-1}$. A further improvement was achieved by applying a conductivity bridge which includes both measuring and reference cell. This makes it possible to directly detect relative variations in impedance due to CO_2, reduces the difficulties of eliminating A-C interference (capacitance effects), and minimizes the influence of temperature changes.

A plot of the specific conductivity against PCO_2 in water is curvilinear and convex to the abscissa. However, in the low range used in the operation of the CO_2 electrode (specific conductivity about 1-3 $\mu S \cdot cm^{-1}$) the curve is practically linear, as shown in figure 6. Position and slope of this straight line depend on the flow rate. Increasing flow rate lowers the specific conductivity at a constant PCO_2 as well as the sensitivity (slope). The reduction of specific conductivity at $PCO_2 = 0$ is due to the decreasing conductivity of the carrier at higher flow rates, probably originating from boundary effects on the surface of the conductivity cell. The sensitivity for CO_2 is diminished at higher flow rates because both contact time of the carrier with CO_2 at the membrane and time available for hydration of CO_2 are shortened. The dependencies of sensitivity and response time on the flow rate are both curvilinear and convex against the coordinates. Thus, high sensitivity and fast response oppose each other. The cell type discussed here at a distance of 10 mm from the membrane provides, when exposed to a step change of PCO_2 between 0 and 5 percent CO_2 and with a flow rate of 0.7 ml/min at 18 °C, a response time of 4 seconds on and 7 seconds off. The sensitivity of this cell in gas or in a stirred liquid phase is similar. The performance of this cell is again summarized in table 1.

Theoretically, the performance of this electrode will depend on the conditions of membrane diffusion, convection, and kinetics of CO_2. In practical terms, the conclusion is important that both flow rate and distance between membrane and cell determine the sensitivity of the system. At constant flow rate, the sensitivity increases with this distance until chemical equilibrium is reached. At constant distance and varying flow rate, the sensitivity is determined by the initial CO_2 concentration in the carrier which is inversely proportional to the flow rate, and by the time required to attain a certain fraction of the equilibrium

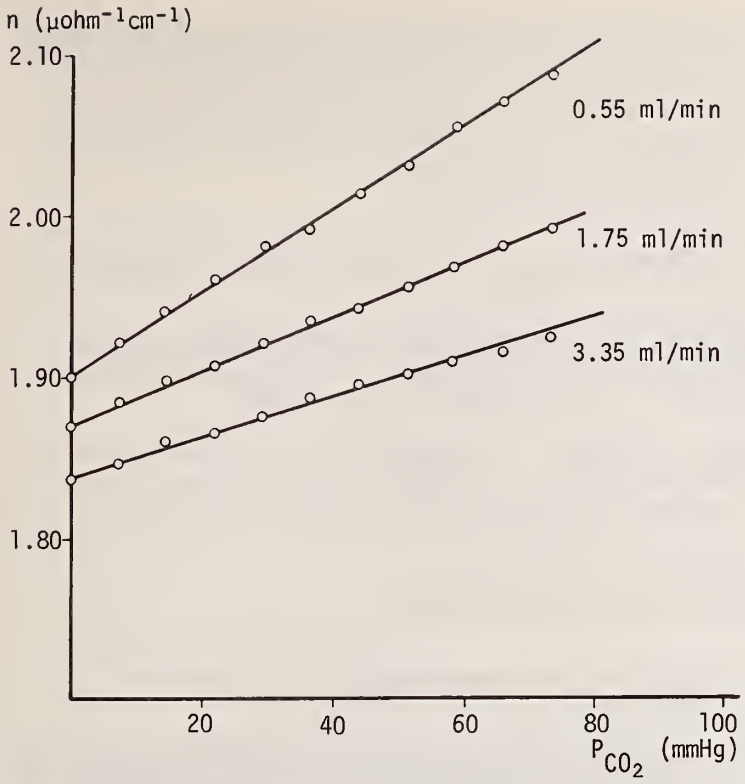

Figure 6. Specific conductivity of the carrier at three different flow rates (0.55, 1.75, and 3.35 ml/min) after exposure of the electrode to a gas phase of varying CO_2 partial pressure at 10 °C [11] (with permission of *Respir. Physiol.*).

concentration and hence on the flow rate as well. When a fast response is required, the conductivity cell should be located close to the membrane. If at the same time high sensitivity and low CO_2 consumption are needed (in order to reduce dependency on the flow rate of the medium), the carrier flow rate may optimally be chosen in the range where turnover of CO_2 into bicarbonate is high. The flow dependency in liquid media might be minimized by using membranes with smaller surface area and/or lower permeability to CO_2. The resulting reduction in sensitivity should then be improved by other means, *e.g.*, by replacement of bidistilled water by highly purified water (conductivity water) which would increase carrier resistance and thus the relative change in specific conductivity. At these lower conductivities, however, balancing of the bridge becomes more difficult which might be partially avoided by using cells of a smaller cell constant. Along the lines of this reasoning, a further miniaturization of the electrode might be expected to increase sensitivity and reduce CO_2 consumption (flow dependency) at the same time.

4. Accurate Estimation of Total CO_2 in Fluids

Finally, it might be mentioned that we have achieved an accurate estimation of total CO_2 in fluids of low CO_2 concentration (0.05-0.5 mmol/l) where the conventional Van Slyke technique with an accuracy of 0.1 mmol/l is likely to produce errors ranging from 20 to 100 percent. The essence of this method [9,12,13] is the use of an infrared CO_2 analyzer (URAS 2, Hartmann and Braun; cell of 250 mm length, corresponding to a full-scale deflection of 0.01 percent CO_2) surrounded by nitrogen rather than by air as usual. The inlet and outlet of the analyzer were connected to a gas extraction chamber provided with a sample inlet system. All connections were made of CO_2 impermeable Viton tubing. The circulating gas flow of 120 ml/min was regulated by a membrane pump and a flowmeter. In order to expel all CO_2 from the sample, 3 ml of 0.1 mol/l lactic acid with antifoam were added to the extraction chamber. Before the injection of the sample, the circuit was flushed with CO_2-free nitrogen and then closed by turning a stopcock. The analyzer was calibrated by mixing various amounts of air (containing 0.03 percent CO_2) with CO_2-free nitrogen in a gas mixing pump. The calibration curve thus obtained is not linear. At these low CO_2 concentrations, water vapor in the closed circuit interferes with the CO_2 readings. This effect of cross-sensitivity could be eliminated by inserting a suitable filter supplied by the firm. In

this way, total CO_2 concentrations of 0.1 mmol/l can be estimated in a 0.5 ml sample with an error of less than 2 percent. By changing the closed circuit volume or the length of the cell, CO_2 concentrations ranging from 0.05 to 100 mmol/l can be estimated without any considerable loss of accuracy. Thiele and van Kempen [14,15] applied this method to the measurement of CO_2 release by human skin and were able to determine CO_2 amounts of ppm/10 cm^2 min.

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NBS STANDARDS FOR pH AND ION ACTIVITY MEASUREMENTS IN BIOLOGICAL FLUIDS

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1. pH Standards

It has been about fifteen years since the National Bureau of Standards, in response to the needs of clinical chemists and physiologists, certified a special composition of the phosphate pH buffer for use in the biological range of interest between pH 7.3 and 7.5 [1][1]. This buffer, with a pH value of 7.392 at 37 °C, has been widely accepted and used in clinical laboratories as the primary pH standard. The proximity of the pH value of this standard to that of blood minimizes errors due to faulty response of the glass electrode or nonlinearity in the meter response. Of course, calibration with a second pH standard such as the 1:1 phosphate buffer (pH = 6.839 at 37 °C) is necessary to detect gross deviations from Nernstian behavior which would necessitate replacement of the glass electrode.

In 1972, NBS reported the availability of a new buffer for use in the physiologic pH range (pH = 7.382 at 37 °C) [2]. This buffer, tris(hydroxymethyl)aminomethane ("Tris") and its hydrochloride salt mixed in a ratio of 1:3 at a tris·HCl molality of 0.05, was selected because it exhibited several advantages over the phosphate buffer. These included stability and compatibility with biological fluids and a temperature coefficient which more closely approximates that of whole blood than does the phosphate buffer [2]. Since preliminary experiments with cells containing a saturated KCl bridge solution and liquid junction indicated residual liquid junction errors (*i.e.*, differences between the operational pH and the corresponding pa_H values) of up to 0.041 pH unit at 25 °C when compared to the 1:1 phosphate buffer [3], the original Tris buffer was issued on a "provisional" certificate until consistency with the pH scale could be checked more thoroughly. This buffer should be considered a secondary standard until more extensive studies of the liquid junction problem are completed.

Ladenson, *et al.* have also noted an inconsistency in the operational pH values when Tris buffers are measured on pH/blood gas analyzers which had been standardized with the phosphate buffers [4]. While their data indicate liquid junction problems with these instruments, the interpretation of their findings is not unequivocal, since they also find pH errors when a physiologic phosphate buffer is measured. The pH errors with the Tris buffers are somewhat larger but also exhibit both positive and negative deviations from the expected values. When directly compared to the NBS phosphate buffer, the Tris buffer gave a pH value which was about 0.012 unit below the assigned value. While the Tris buffer may not be suitable for use as a primary standard, Ladenson, *et al.* found it is eminently suited for comparative measurement with blood pH systems. It also proved to be convenient and effective in monitoring the performance of these analyzers.

[1]Figures in brackets indicate literature references at the end of this paper.

In order to better approximate the colligative properties of blood plasma and serum, preliminary investigations have been made into the certification of the Tris buffer in isotonic saline solution [2]. While it was hoped that this expedient would reduce the uncompensated residual liquid junction potential, initial studies at NBS [3] again indicate operational pH values that are low by about 0.05 pH unit at 25 °C when measured in a cell with a saturated KCl liquid junction. It is obvious that much more extensive research must be carried out to elucidate the nature and magnitude of this problem.

Whereas the Tris buffer may eventually prove to be unsuitable as a pH standard, there are many other possible materials for use as clinical pH buffers, such as the materials in the list published in 1966 by Good, *et al.* [5]. One of these, tris(hydroxymethyl)methylglycine ("Tricine"), was the subject of a recent study by Bates and coworkers [6] who found that a solution consisting of 0.06 m Tricine + 0.02 m sodium Tricinate (pa_H = 7.407 at 37 °C) had a residual liquid junction error of about 0.01 pH unit which would not exclude the Tricine buffer from consideration as a primary standard for pH measurements. Studies in isotonic saline solution of two other zwitterionic buffers, HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid] and TES [N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], with pH values between 7 and 8 have recently been completed by C. A. Vega at the University of Florida.

2. Ion Activity Standards

Ion-selective electrodes, like the pH glass electrode, are finding growing use in various biomedical studies for determining or monitoring the activities of ions such as Ca^{++}, Na^+, K^+, Cl^-, F^-, etc. One very important area of development is the use of these electrodes in automatic, multi-electrolyte clinical analyzers. As in the case of the pH electrode, standards are also required for the reliable application of these sensors [7]. Investigations at NBS have been primarily concerned with aqueous standards and will progress in the future to mixed electrolyte systems and synthetic biological fluids. To date, NBS has certified standards for Na^+, K^+, Cl^-, and F^-, and a standard for Ca^{++} is in progress.

In considering the certification of ion activity standards, one important difference exists in regard to the measurement process. For pH certification, an electrode system of proven reversibility exists, *i.e.*, the hydrogen gas electrode/silver-silver chloride electrode, whereas in the case of ion-selective electrodes, the ideality of behavior has not been unequivocably demonstrated. This consideration requires that more reliance be placed on the activity scales and theoretical conventions used in calculating the single-ion activities. The NBS has adopted the convention for single ionic activities proposed by Bates, *et al.* [8] which is based on the hydration theory of Stokes and Robinson [9]. The details of this ion activity scale have been reviewed by Bates [10] and will not be repeated here. Suffice it to say that the single ion activity coefficients are calculated from experimental values of the mean molal activity coefficient (γ_{MX}), the osmotic coefficient (ϕ), and a hydration number (h) according to the equations for univalent electrolytes:

$$\log \gamma_{M^+} = \log \gamma_{MX} + 0.00782\,(h_M - h_X)m\,\phi$$

and

$$\log \gamma_{X^-} = \log \gamma_{MX} + 0.00782\,(h_X - h_M)m\,\phi.$$

As in the case of the pH scale, it is necessary to make certain extra-thermodynamic assumptions. The "conventional" step in the hydration treatment is the assignment of hydration numbers to individual ionic species. The hydration numbers are calculated from smoothed values of γ_{MX} and ϕ using the Stokes-Robinson hydration theory. The hydration number for the electrolyte is then "split" between the cation and anion by assigning a hydration value to one of the ions. For the present scale, the hydration number for the chloride ion was taken to be zero, and the other ionic hydration numbers are referred to this convention [10]. Ionic activity for unassociated electrolytes derived on the basis of this hydration convention have been found to be consistent with the observed responses of ion-selective electrodes.

Whereas the ion activity standards certified at NBS to date have been single salts intended for use in relatively simple electrolyte solutions, such standards are only reliable over a concentration range of three or four decades, due to ion association problems at higher concentrations and contamination and/or adsorption problems at lower concentrations. One way to avoid this latter difficulty is to prepare ionic activity buffers which can extend the low activity limit by several orders of magnitude.

Early studies at NBS demonstrated that the linear response of the silver ion-selective electrode could be extended down to approximately $10^{-2.5}$ mol/l with solutions consisting of mixed silver halides and sulfide [11]. Not only was the response linear and approximately Nernstian over 25 decades of concentration, but whereas the response time of the electrode in unbuffered silver ion solutions increased with decreasing concentration, in the silver-buffered solutions, the electrode response was almost immediate, *i.e.*, less than 15 seconds, even at the lowest levels of silver ion activity.

While the concept of "metal buffers" is not new, their use in the calibration of ion-selective electrodes has been studied primarily by workers in Denmark and Hungary [12-17]. In Denmark, Blum and Fog developed water-soluble buffers for copper which avoid the slowness of solution equilibration inherent in precipitate-type buffers which involve heterogeneous equilibria [12]. This concept has been extended by Hansen, Ruzicka and coworkers [13-15]. They calibrated ion-selective electrodes using buffers for Cu^{++}, Cd^{++}, and Ca^{++} based on soluble EDTA and NTA complexes.

In Hungary, Havas, Kaszas, and Varsanyi have reported [16] the development of mixed precipitate buffers for the halides (including fluoride) and silver ion. More recently, Bailey and Pungor have suggested that, instead of serial dilutions of standards, the standards in the range from 10^{-4} to 10^{-7} mol/l should be prepared by electrolytic generation of the appropriate ion [17]. They demonstrated their technique with iodide and silver and also indicated that sulfide, fluoride and thiocyanate could be generated electrochemically. Although the solutions are not buffered, an advantage of such a procedure is that it could be automated and is more reproducible than the serial dilution method.

Great care must be exercised, however, in applying either the "pure" electrolyte or buffered standards to the calibration of ion-selective electrodes below the point where the pure electrolyte response begins to deviate from linearity. The emf *vs.* ion activity response will be entirely different for the two types of standards, and the interpretation of the sample data will depend upon whether the sample system is buffered or not. It is clear that much more work is required to develop ion activity standards which will be suitable for most applications and acceptable to everyone.

3. Standards for the Future

As the requirements of clinical laboratories and available instrumentation become more refined, data for salt effects and medium effects on electrolytes and gases are needed to provide the basis for the development and certification of the required standards and quality control materials.

We plan to obtain data for ionic equilibrium processes in saline media, to develop accurate methods for determining ion concentrations and gas tensions, and to develop multi-component standards for calibration of the clinical instruments used for the determination of pH, dissolved gases, and electrolytes in various biological fluids.

Ultimately such standards may contain all of the major ionic constituents, complexing agents, and non-electrolytes at levels approximating those in the real samples. Thus, a synthetic blood standard would consist of an isotonic electrolyte solution containing, for example, Na^+, K^+, Ca^{++}, Mg^{++}, O_2, HCO_3^-, Cl^-, $HPO_4^=$, and polyelectrolytes in physiological amounts. Of course, the problems encountered in developing such a multicomponent standard would be very great, but the availability of a single standard for the calibration of instrumentation for the determination of multiple parameters is vitally needed for the future development of this field. Only when such standards are available will it be feasible to calibrate multi-parameter clinical instruments conveniently and with some assurance of the consistency and comparability of data obtained in different laboratories. As far as the major ionic constituents are concerned, the first step in this direction has already been taken [18].

We are optimistic that given a high enough priority and sufficient time, the problems will be solved. The continued support of organizations such as those sponsoring this workshop will help insure the success of this goal.

Addendum

NBS Facilities for pH Certification Measurements

Since there was no time during the Workshop on pH and Blood Gases for the participants to tour the facilities used for electrolyte studies and pH certification, a section from a recent NBS Special Publication [19] on this subject is being reprinted here.

In 1972, the NBS emf measurement system was automated in order to increase the reliability of the data acquisition and to reduce the time required for a pH certification run from about five 8-hour days to two 24-hour days with minimal operator attention. The primary purpose of this system is to cause a constant temperature water bath to cycle through a preprogrammed set of temperatures and to record the data when the bath temperature and potentials read from the measurement cells pass certain stability and control requirements.

The temperature-controlled emf measurement system is diagrammed in figure 1. A centrally located computer, a Univac Series 60, model 6135, is time-shared with about a dozen other systems in the Analytical Chemistry Division. The data communications system consists of four main parts: (1) the digital data bus, (2) the computer interface, (3) the laboratory logic box interface, and (4) the laboratory control console. The computer interface connects the computer to the laboratory via the digital data bus and laboratory interface. The laboratory instrument console provides control and the capability of entering auxiliary data for use by the computer to service and/or control the interfaced system. Two-party line digital data busses have been installed in the Chemistry Building with outlets for each

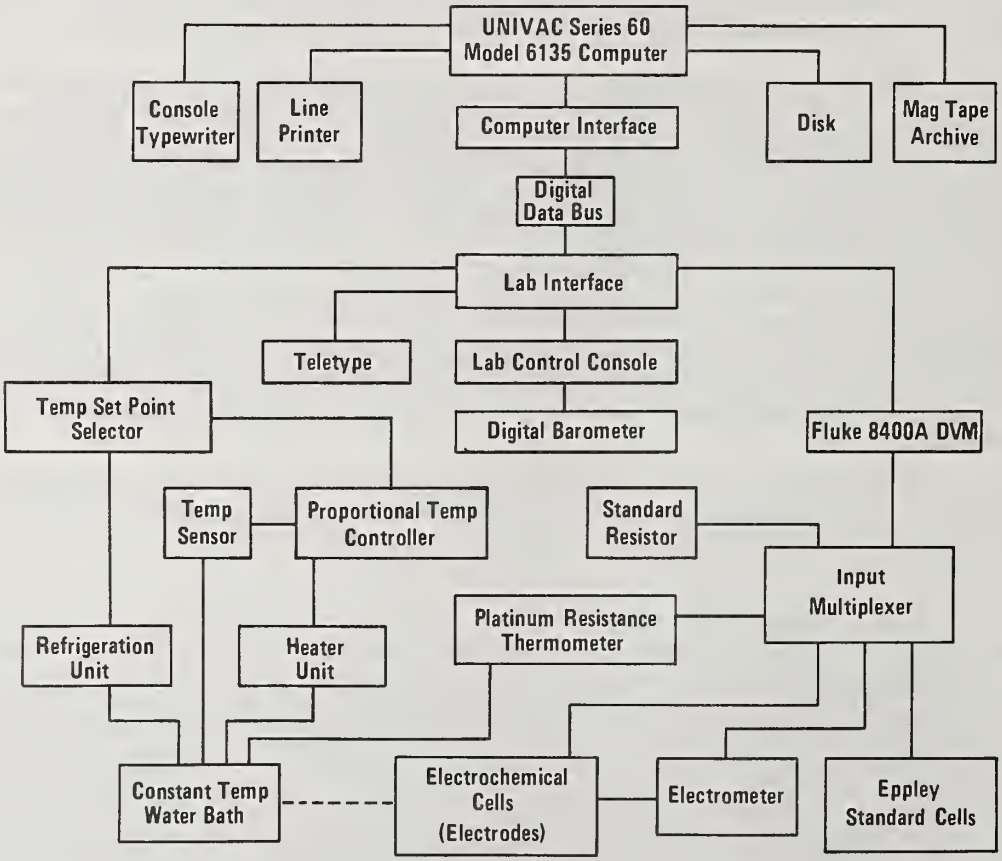

Figure 1. Diagram of the temperature-controlled emf measurement system.

laboratory. The communication between the laboratory and the computer interfaces is accomplished by a multi-level time-sharing scheme. The laboratory interface contains the logic circuitry necessary to connect as many as four experimental systems to the digital data bus. Data that are routinely collected by the system are stored on a random-access disc for a period of several days. If space on the disc becomes scarce, the data files are read onto a master archive magnetic tape for storage.

In performing a pH certification, the first requirement is to prepare the buffer solutions and measurement cells with utmost care. As specified in the pH certificates, the carefully dried and weighed salts are dissolved in distilled water of sufficient purity to have a conductivity of less than 2×10^{-6} $S \cdot cm^{-1}$ ($ohm^{-1} \cdot cm^{-1}$) at 25 °C. For buffers in the neutral and basic regions, carbon dioxide must also be removed from the water prior to dissolution of the buffer salts. The hydrogen gas is purified of oxygen by passage through a catalytic reduction tube or through a palladium purifier.

The specially designed emf measurement cell shown in figure 2 consists of a hydrogen gas electrode compartment, a silver/silver chloride electrode compartment, and a series of gas-dispersion compartments for humidifying the incoming hydrogen gas by passage through the buffer solution. Details of the preparation of the platinized (or palladized) platinum and the silver/silver chloride electrodes can be found in references [20] and [21].

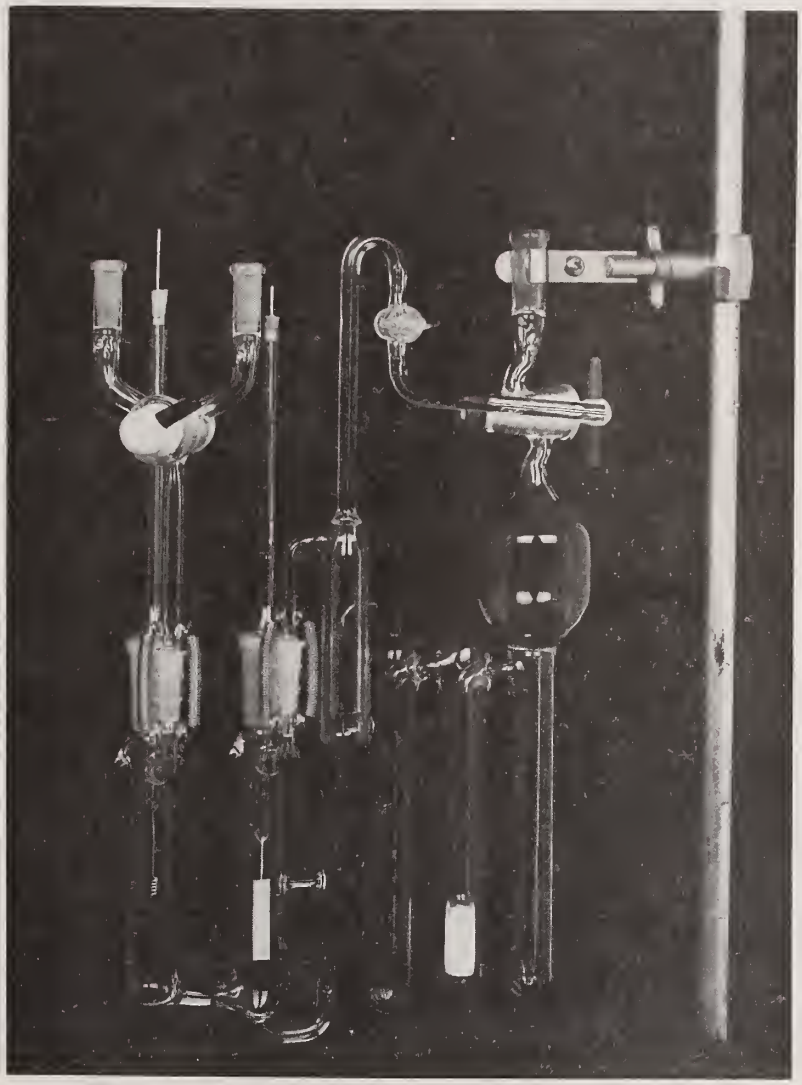

Figure 2. Emf measurement cell used for the certification of pH buffer solutions.

The prepared measurement cells, usually consisting of two cells containing each of the three levels of added chloride, are placed in the controlled temperature water bath as shown in figure 3. Also shown in this figure are the coiled copper tubing hydrogen gas inlets and the two platinum resistance thermometers.

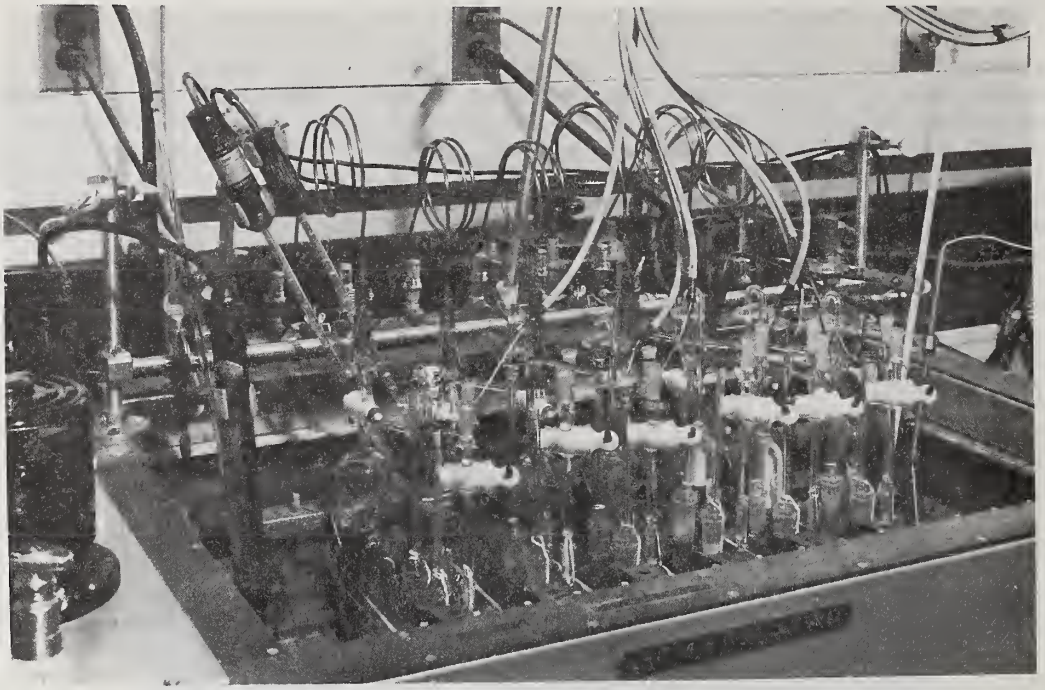

Figure 3. Emf measurement cells in position in the controlled-temperature water bath.

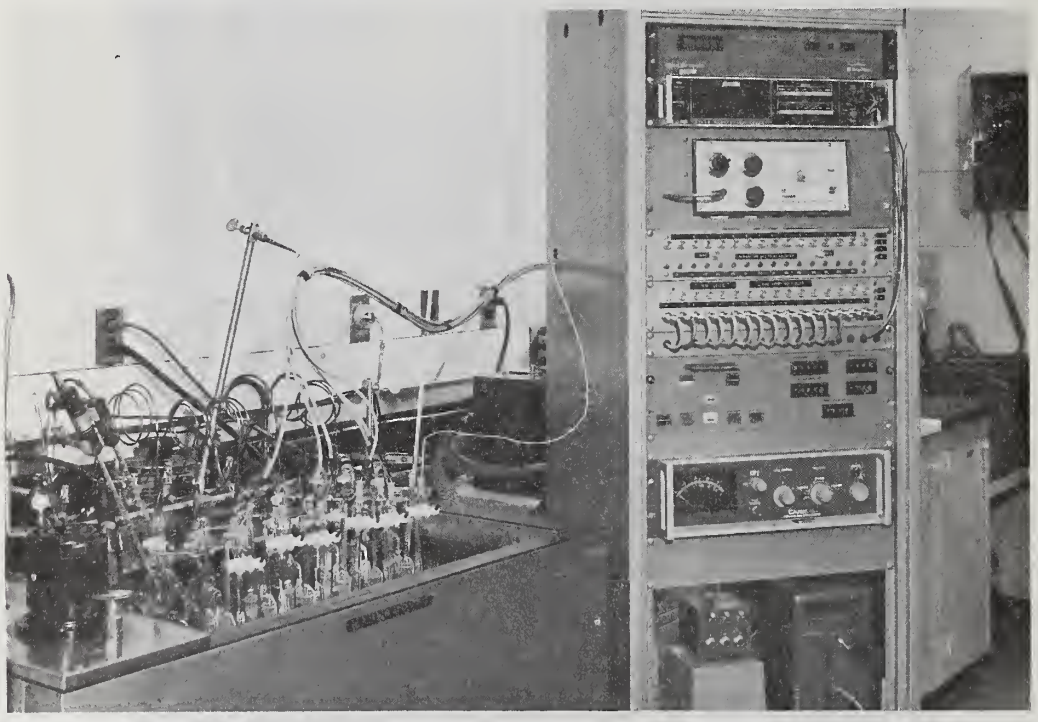

Figure 4. View of the measurement facility including the water bath, instrument rack and laboratory logic box interface (open on rear wall).

The complete measurement system, with the laboratory logic box interface (open) on the rear wall, is shown in figure 4. Another view, figure 5, shows the entire electronics rack and the thermostated standard cells on the laboratory bench top. Briefly, the equipment in the electronics rack consists of (from the top): A digital barometer which is automatically

Figure 5. Close-up view of the instrument rack with thermostated standard cells on the laboratory bench to the right.

monitored during the certification measurements is used to correct the partial pressure of hydrogen for variations in the atmospheric pressure; the autoranging and autofunctioning digital voltmeter which serves as the analog-to-digital converter for both resistance and emf measurements; the proportional temperature controller which, in conjunction with the temperature set point selector (next panel down) and the bath refrigeration unit, maintains preset bath temperatures to better than ± 0.01 °C by means of immersion heaters; and the DVM input multiplexer which, under computer control, switches the various measurement parameters into the DVM for A-to-D conversion prior to transmission to the computer for acquisition and storage. The functions multiplexed include: temperature (platinum resistance), standard resistor (calibration), standard emf cells (calibration), and up to ten measurement cells. The control console permits communication with the computer *via* a series of thumbwheel switches which enter all of the required input parameters, *e.g.*, temperature sequence, number of cells, time delays, and program identification numbers. The control console also includes a set of pushbuttons which are used to set up, start (send), and terminate the experiment, as well as indicator lights which signal operations (data acquisition, operate, auto, *etc.*) or problems (error, reject). A vibrating-reed electrometer is located below the control console and, in conjunction with the three-position pH switch, provides a high-impedance input to the DVM for measurements with glass and high-resistance ion-selective electrodes. The remainder of the equipment in the rack constitutes the Mueller bridge system for manual checking of the water bath temperature via the second platinum resistance thermometer shown in figure 3.

In operation, computer control begins by automatically positioning the temperature set point selector at the starting sequence number. When the bath temperature achieves the nominal value, within certain preset control limits as indicated by the platinum resistance thermometer, the input multiplexer automatically switches through the readout positions. After a programmed time delay, the multiplexer recycles through the positions and compares the values to the previous sets of data. After three cycles, if the data agree within the requisite control limits, the computer sets the temperature control to the next sequence value and the operation is repeated. If any of the values exceed the control limits, an out-of-range message is printed next to the incorrect value and, following two more cycles, the computer proceeds to the next sequence temperature after again flagging the out-of-range value. Depending on the temperature value or the direction of the temperature change, the computer turns on the refrigeration unit or an auxiliary heater. After the temperature sequence is completed, the routine is automatically terminated.

Reduction of the acquired data is performed by batch operation on the NBS central computer, a Univac 1108. The data reduction consists of a series of operations including correcting the experimental emf values to the standard partial pressure of hydrogen, calculating the acidity functions, extrapolating to zero molality of added chloride to obtain the limiting acidity functions, evaluating the chloride ion activity coefficient using the Bates-Guggenheim pH convention, and finally calculating the pa_H values. These experimental values are then smoothed with respect to temperature by the method of least squares to give the certified pH(S) values.

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USE OF CARBON DIOXIDE- AND OXYGEN TONOMETERED PHOSPHATE-BICARBONATE-CHLORIDE-GLYCEROL-WATER MIXTURES FOR CALIBRATION AND CONTROL OF pH, pCO_2, AND pO_2 ELECTRODE SYSTEMS

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The desire for simultaneous calibration of pH, pCO_2, and pO_2 electrode systems of modern blood gas analyzers and the need for adequate quality control for this equipment stimulated this investigation. The most important difficulties to overcome were: (1) establishing the pH, pCO_2-relationship of the calibration solution, and (2) systematic bias between the response of gas electrodes, in different media.

1. Calibration Solutions

In this study (for details see reference [1][2]), we started from phosphate-bicarbonate-chloride solutions equilibrated with carbon dioxide gas for which we were able to calculate a pH, log pCO_2-relationship based on the law of electroneutrality and the definition of the ionic strength, using the practical ionization coefficients of phosphoric acid from Bates [2], and of carbonic acid from Maas [3]. For the NBS equimolal phosphate buffer (0.025 mol/l) to which $NaHCO_3$ and NaCl were added in a total concentration of 0.060 mol/l, we found in the pH range 6-8, at 37 °C the following equation set:

$$pH = 7.1834 - 1.34207\sqrt{I_c} + 0.8436 I_c + a - \log \frac{0.185 - I_c}{I_c - 0.135} \quad (1)$$

$$pH = 10.806 - 0.4662\sqrt{I_c} + \alpha - \log pCO_2 + \log (0.160 - I_c + y) \quad (2)$$

in which I_c is the ionic strength on a concentration basis, y the added amount of $NaHCO_3$ and α the factor wherein the deviation of the practical ionization coefficients defined in other ionic and medium systems is discounted.

Subtracting eq (2) from eq (1) results, after rearrangement, in a relationship between pCO_2 and I_c:

$$pCO_2 + \frac{(0.185 - I_c)(0.160 - I_c + y)}{(I_c - 0.135)} \times 10^{(3.6226 + 0.8758\sqrt{I_c} - 0.8436 I_c)}. \quad (3)$$

This means: by equilibration of buffer solutions of this kind, with gas mixtures of known composition, that is to say a given pCO_2, the ionic strength I_c is fixed, and could be estimated by eq. (3). In this manner, pH-log pCO_2 equilibration lines of buffer solutions

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[2]Figures in brackets indicate literature references at the end of this paper.

with different sodium bicarbonate concentration can be predicted from eqs. (1) and (2), on the condition that α is known. The factor α could be determined from eq. (1) or (2) by pH measurements of equilibrated buffer solutions at a given pCO_2. From figures 1 and 2, it is demonstrated evidently that introduction of an empirical factor α leads to a good agreement.

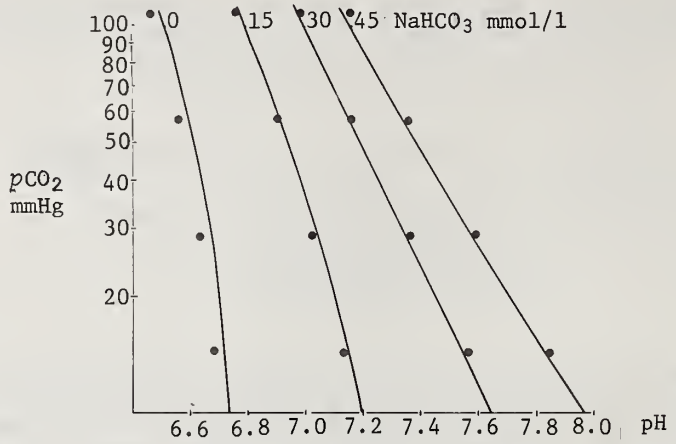

Figure 1. pH-log pCO_2 equilibration lines of four buffer solutions with 0, 15, 30 and 45 mmol/l sodium bicarbonate, respectively, calculated by means of eqs. (1) and (2) without correction factor α; the dots are the measured pH values of the same buffer solutions equilibrated with gas mixtures of 2, 4, 8 and 15 percent CO_2, respectively.

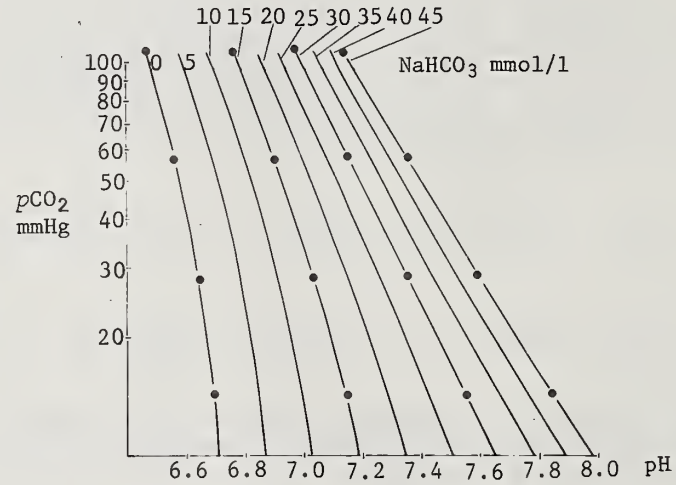

Figure 2. pH-log pCO_2 equilibration lines, differing from each other by 5 mmol of sodium bicarbonate per liter, calculated from eqs. (1) and (2) by substituting α. The dots are the same measuring points as in figure 1.

It is known from the literature [4] that whole blood and glycerol/water (3/7 by volume) mixtures at the same oxygen tension produce nearly equal diffusion currents as a consequence of their similar viscosity. In order to minimize differences between blood samples and calibration solutions, we added glycerol to the buffer solutions. In aqueous solutions the correction term (α) was dependent on y:

$$\alpha = 0.0331 + 1.513y - 8.44y^2 - 118.5y^3. \quad (4)$$

In the glycerol/water (3/7 by volume) solutions, the correction term (β) was dependent on

I_c as well, caused by the medium effect:

$$\beta = (-5.667 + 290.999y - 3113.55y^2 - 4573y^3)I_c + 0.8700 - 39.343y + 317.56y^2 - 2459.3y^3. \quad (5)$$

By means of eq. (5) substituted in eqs. (1) and (2) (read $\alpha = \beta$), pH-log pCO_2 equilibration lines of glycerol/water (3/7 by volume) buffer solutions with different sodium bicarbonate concentration can be predicted.

So we were able to prepare suitable calibration solutions of desired pH, pCO_2 and pO_2 values, by equilibrating a solution of 25 mmol of the phosphates, 30 mmol sodium bicarbonate and 30 mmol sodium chloride per liter glycerol/water (3/7 by volume) mixture with 4 percent CO_2 in air and 8 percent CO_2 in nitrogen, respectively: Various pH, pCO_2, and pO_2 values, depending on the barometric pressures, are given in the next calibration table 1.

Table 1. Calibration values obtained by equilibration of above mentioned buffer solution gas mixtures 1 and 2 at 37 °C over the barometric pressure (B) range 740-780 mmHg.

	Gas mixture 1 4% CO_2 and 20.09% O_2			Gas mixture 2 8% CO_2 and 0% O_2		
B	pH	p_{CO_2}	p_{O_2}	pH	p_{CO_2}	p_{O_2}
mmHg		mmHg	mmHg		mmHg	mmHg
740	7.420	27.7	139.2	7.226	55.4	0.0
745	7.418	27.9	140.2	7.224	55.8	0.0
750	7.416	28.1	141.2	7.222	56.2	0.0
755	7.414	28.3	142.2	7.220	56.6	0.0
760	7.412	28.5	143.2	7.218	57.0	0.0
765	7.410	28.7	144.3	7.216	57.4	0.0
770	7.408	28.9	145.3	7.214	57.8	0.0
775	7.406	29.1	146.3	7.212	58.2	0.0
780	7.404	29.3	147.3	7.210	58.6	0.0

The usefulness of this calibration buffer system was checked by measuring pH, pCO_2 and pO_2 values of tonometered whole blood on the Corning M 165 blood-gas analyzer, both by the conventional calibration methods (buffer and gases) and by using the equilibrated solutions. The measurements of pH and pCO_2 were at least comparable, whereas pO_2 measurements were substantially improved by minimizing the gas-blood difference using calibration liquid of broadly the same viscosity as blood (see fig. 3).

The accuracy of this calibration method depends on the accuracy of the phosphate buffers, against which the pH values of the calibration solution is operationally derived (±0.005 pH), and the accuracy of the composition of the gas mixtures (±1% of the pCO_2 and pO_2 value).

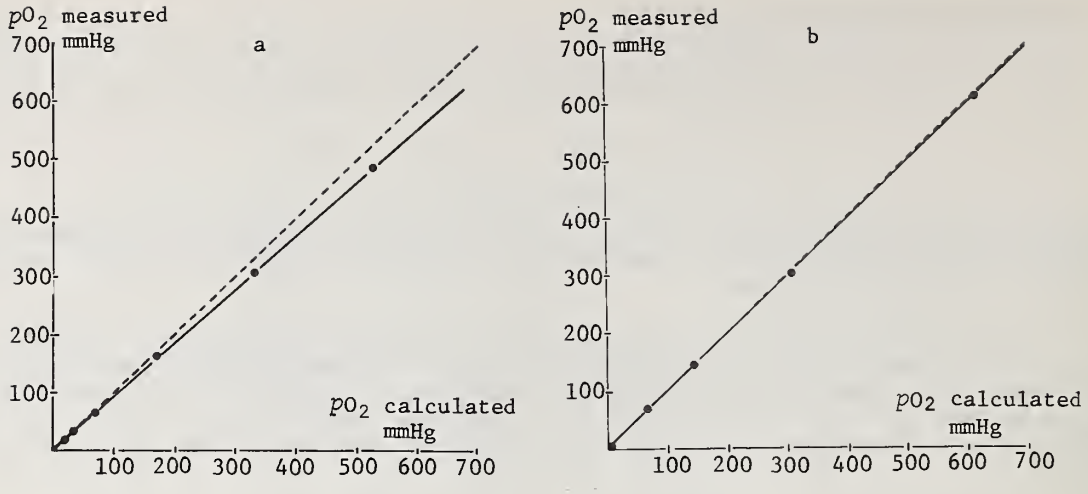

Figure 3. Relationship between measured pO_2 and calculated pO_2. *Continuous lines* are the calculated regression lines for y on x and the *broken lines* are the lines of identity; (a) gas-calibration method; (b) buffer-calibration method.

2. Approach to Quality Control

The proposed buffer system not only opens new perspectives for simultaneous calibration of pH, pCO_2, and pO_2 electrodes, but it also is a step in the direction of quality control, as indicated by Noonan and Burnett [5]. Realization can be thought by the following line:

(a) Intralaboratory comparison of blood gas equipment can easily be realized by offering samples from the same thermostated tonometer setup, to the different apparatus.

(b) Interlaboratory comparison would be possible in principal by sending around ampoules containing a control solution for simultaneous control of pH, pCO_2, and pO_2.

In our routine laboratory, we have at our disposal three blood-gas apparatus: from Radiometer, Models BMS2-MK2, and ABL-1; from Instrumentation Laboratory, Model IL-413. For quality control of the BMS2-MK2, we measure a few times a week the pH of buffer solutions, marked A III and C III (composition: 0.025 mol/liter Na_2HPO_4, 0.025 mol/liter KH_2PO_4, 0.030 mol/liter $NaHCO_3$ and 0.030 mol/liter NaCl using, respectively, water (A III) and glycerol/water (3/7 by volume) mixture (C III) as solvent) tonometered with gas mixtures of 4 percent CO_2 and 8 percent CO_2 in air. In figure 4, the fluctuation of the bias between the readings and the stated values over a two-month period is presented.

We also obtained initial experience with quality control of the ABL-1 and IL-413 with the same buffer solutions equilibrated in a special setup consisting of one gas mixing pump, delivering two gas mixtures, and a thermostated tonometer vessel. The results of twelve measurements performed on various days are summarized in table 2.

The pH differences (*ca.* 0.012 pH) could be explained partly by a difference of 0.008 pH between the ABL-1 and IL-413 calibration buffers and the Radiometer precision buffer (pH = 7.383), and partly by the type of salt bridge: ABL-1, 20 percent KCl; IL-413, saturated KCl with a membrane instead of an open junction.

The pCO_2 differences (table 2) are equal for both instruments and do not deviate significantly from the stated value for the lower pCO_2 value in contrast to the measured high pCO_2 values which are *ca.* 3-5 percent lower. Comparison of results obtained with both instruments using tonometered blood samples revealed a relationship between the measured and calculated pCO_2 values as presented in figures 5a and 5b. The regression lines cross the identity lines near the lower calibration point. The main cause of deviation from identity seems due to the "memory effect" of pCO_2 electrodes as recently described by Berkenbosch [6], Cramption Smith [7], and Hahn [8]. They show also that this effect is reproducible and corrections can be made for it.

Table 2. Difference between measured and stated values of equilibrated buffer solutions A III and C III obtained with the blood gas analysers. Models ABL-1 and IL-413.

Buffer solution	A III				C III			
Gas composition	5% CO_2, 19.88% O_2		10% CO_2, 0% O_2		5% CO_2, 19.88% O_2		10% CO_2, 0% O_2	
Started values at Bar = 760 mmHg	pH = 7.285 pCO_2 = 35.6 mmHg pO_2 = 141.3 mmHg		pH = 7.092 pCO_2 = 71.3 mmHg pO_2 = 0 mmHg		pH = 7.350 pCO_2 = 35.6 mmHg pO_2 = 141.3 mmHg		pH = 7.155 pCO_2 = 71.3 mmHg pO_2 = 0 mmHg	
Apparatus	ABL-1	IL-413	ABL-1	IL-413	ABL-1	IL-413	ABL-1	IL-413
ΔpH	- 0.010	- 0.011	- 0.009	- 0.011	- 0.014	- 0.015	- 0.015	- 0.012
SD	0.004	0.004	0.004	0.004	0.003	0.005	0.004	0.003
ΔpCO_2 (mmHg)	- 0.3	+ 0.1	- 1.5	- 3.5	- 0.3	+ 0.7	- 2.2	- 3.9
SD	0.4	0.7	1.5	1.1	0.7	0.7	0.6	1.4
ΔpCO_2 (%)	- 0.9	0.2	- 2.6	- 4.8	- 0.9	2.0	- 3.0	- 5.5
SD	1.2	1.8	1.5	1.5	1.9	1.9	0.8	1.9
ΔpO_2 (mmHg)	- 1.0	- 6.4	27.3	28.2	- 9.0	- 7.3	26.0	30.8
SD	2.2	2.6	6.0	11.7	1.0	1.7	10.3	7.3
ΔpO_2 (%)	- 0.9	- 4.5			- 6.3	- 5.1		
SD	1.5	1.8			0.7	1.2		

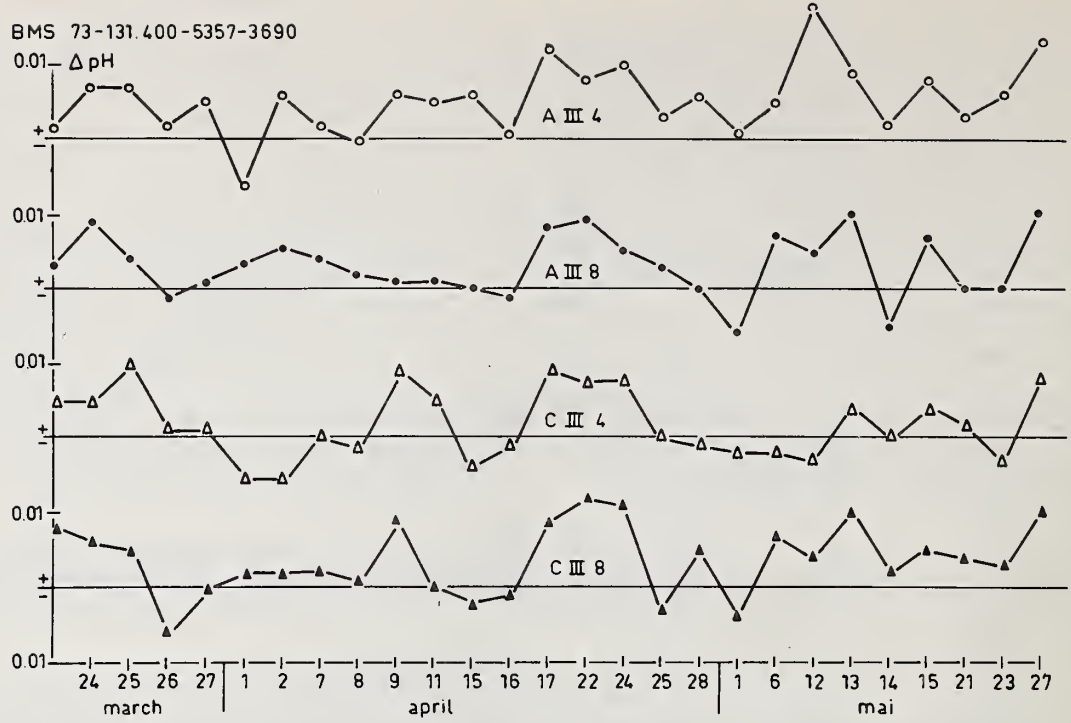

Figure 4. Tonometry results for pH, with BMS2-MK2 (see text). The mean differences and standard deviations are: A III 4: 0.0054 ± 0.0045; A III 8: 0.0030 ± 0.0044; C III 4: 0.0026 ± 0.0046; C III 8: 0.0040 ± 0.0049 which means that the precision is very high.

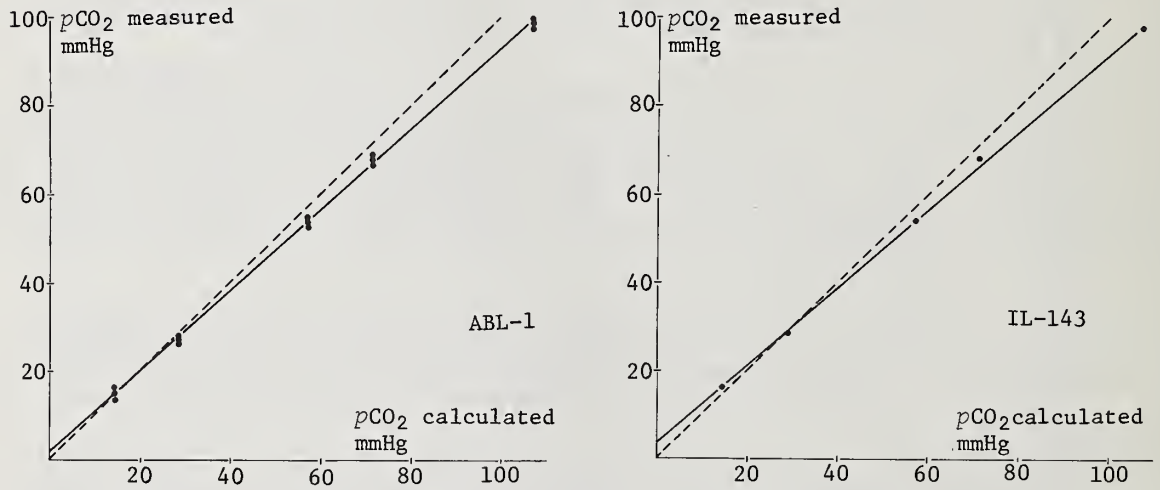

Figure 5. Relationship between pCO_2 of blood and gas: (a) measurements with ABL-1; y = 0.9115x + 2.1091; (b) measurements with IL-413; y = 0.8822x + 4.2134. Each dot is the mean of ten measurements.

Concerning the pO_2 measurements (table 2), we found that the zero point could not adequately be established by a single filling of the cuvette probably caused by mixing with contaminants remaining behind in the chamber after a measurement and oxygen loss from the electrode. Values of *ca.* 30 mm Hg were read.

Using A III solution, the high pO_2 values agree with the stated value for the ABL-1 and were significantly lower for the IL-413; using C III solution, the pO_2 readings are lower for both instruments. Also we evaluated the pO_2 electrode systems of both instruments using tonometered blood samples. The results are illustrated in figures 6a and 6b. There is a tendency for the blood-gas regression lines to have slopes lower than those due to gas for both instruments. In the physiological range the difference between the value for gas and tonometered blood is a few percent, but at higher oxygen tension readings are significantly too low. We feel that there is a need to improve the method of calibration of blood-gas analyzers.

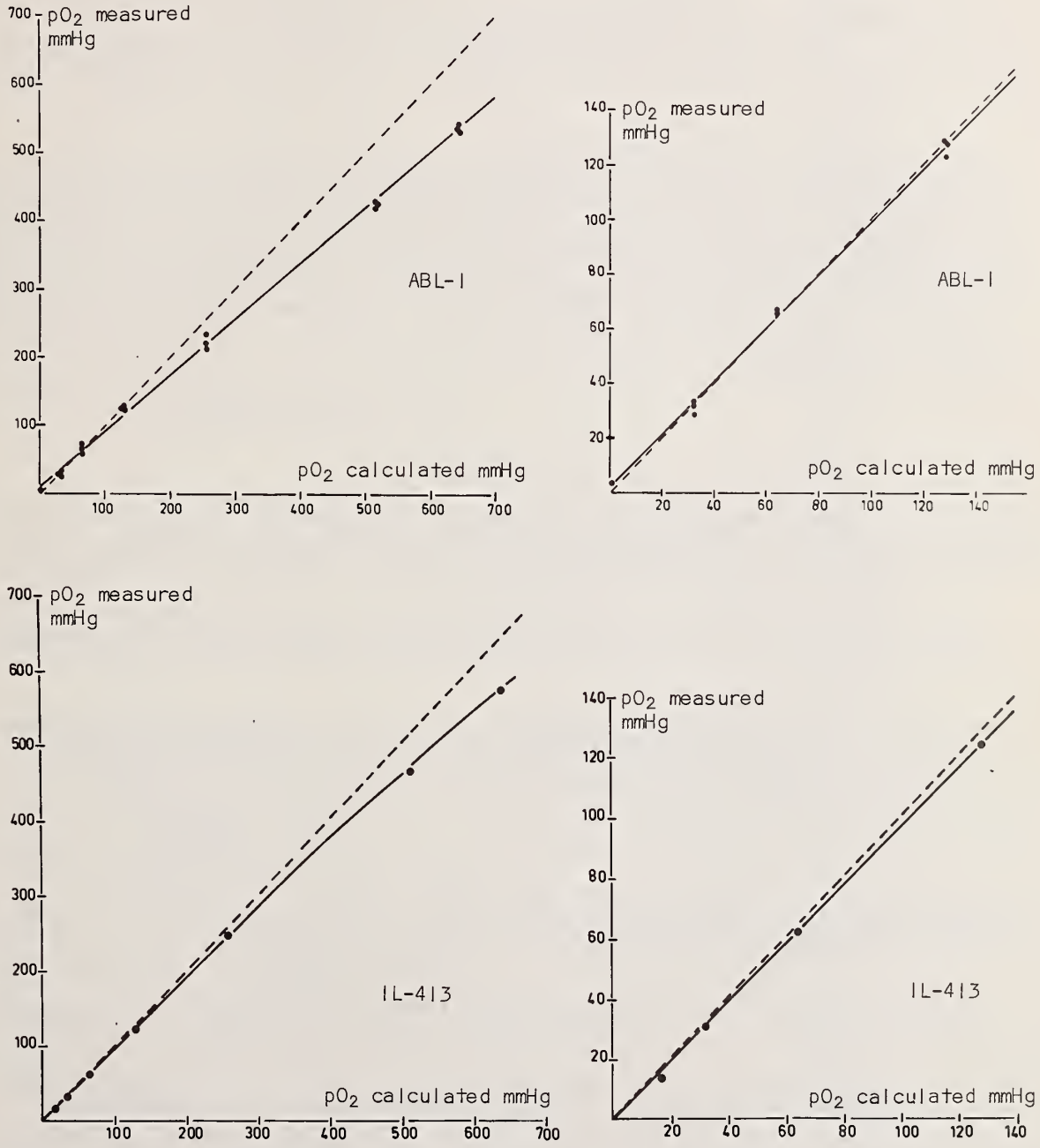

Figure 6. Relationship between pO_2 of blood and gas: (a) measurements with ABL-1; $y = 0.9682 + 2.2981$; (b) measurements with IL-413; $y = 0.9804 - 1.0731$. Each dot is the mean of ten measurements.

Introductory in two trials, we have sent around 100 ml buffer solutions in plastic bottles to approximately ten laboratories with the suggestion to equilibrate these solutions with gas mixtures of CO_2 content and to measure pH. So this method was restricted to setups of the indirect pCO_2 method according to Astrup and Siggaard-Andersen. In figure 7 the results of these trials are reflected.

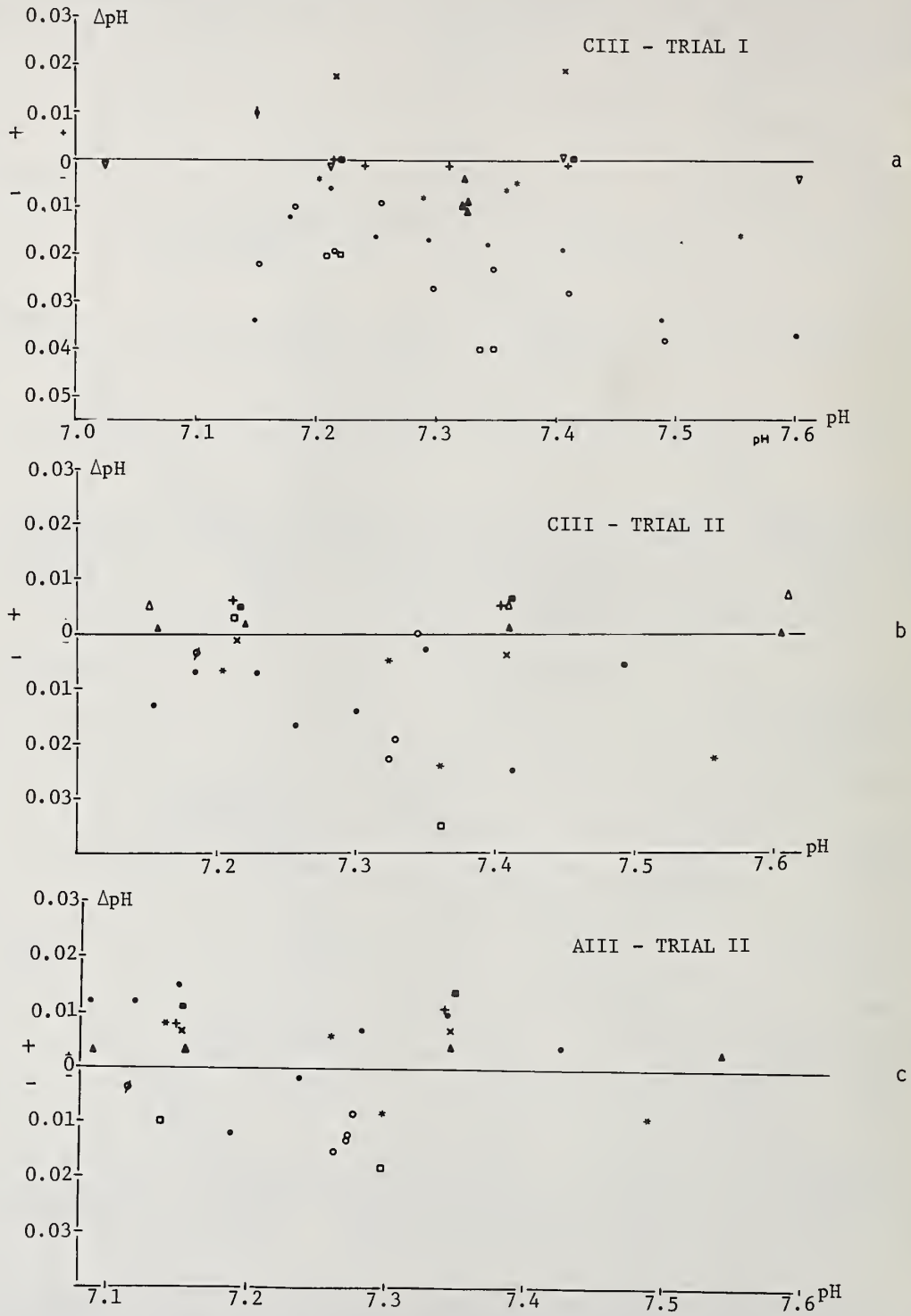

Figure 7. Two trial results of pH determined in CO_2 equilibrated A III and C III buffer solutions. Different symbols are from different laboratories.

In reference to these figures, we may make the following remarks:

(1) Rather good agreement (*ca.* 0.01 pH) in the physiological pH range is found by most of the participants.

(2) Improvement of the results at the second trial, probably caused by experience and sending around a precision phosphate buffer for calibration as well. Dramatic was the discovery of pH deviation of 0.011 unit between the precision buffer sent along, and a buffer from the same firm and with the same batch number, present in one of the laboratories. Up to now the explanatory suggestions are still unproved.

(3) Differences up to 0.02 pH unit for equipment in the same laboratory. See the closed and open squares in figure 7a.

(4) Possibility of signaling a wrong analysis of a gas cylinder. See open square at the bottom of figure 7b.

(5) Possibility of detecting small difference in gas mixing pumps. See open and closed triangles in figure 7b.

In an attempt to eliminate differences in the equilibration setup, and to simplify the procedure, we are engaged in an investigation into the possibility of additives to the buffer solution, which are able to absorb oxygen. To such a solution, with enhanced capacity, the desired carbon dioxide and oxygen tension can be introduced. The only thing to be done, prior to pH, pCO_2 and pO_2 measurement, is to bring the solution to 37 °C before opening the sample holder. Preliminary results with a fluorocarbon, because of its high dissolving capacity for gases, showed usefulness for some equipment and uselessness for others.

Most likely there will still be many obstacles on the way to quality control, however, the purpose is worthwhile continuing this way.

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CALIBRATION OF BLOOD GAS ANALYZERS

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Blood gas measurements are unique in the field of medical laboratory analysis. They are frequently requested at a point when the subject patient is in a life-threatening situation which will be dealt with according to the results obtained from these measurements. The techniques that are used by experienced blood gas technicians for collection and storage of samples and carrying out of measurements are complex but highly standardized: anaerobic sample handling, "double insertion" of the sample into the cuvette, monitoring of cuvette temperature and waiting for the proper end-point to be achieved are good examples of the precise operator technique required. The equipment used by virtually every medical laboratory to perform blood gas analysis is basically identical (glass electrode system for pH, Severinghaus electrode for PCO_2 and Clark electrode for PO_2), with variations in the volume of blood sample required for analysis, design of supporting hardware and degree of automation. Yet, for all the similarities in the methodology for measuring blood gas, the means for calibration of blood gas analyzers are not uniform.

Classically, blood gas measurements have been calibrated using dilute aqueous phosphate buffers for pH [1][1] and analyzed gases for PCO_2 and PO_2 [2]. Many modifications of calibrating materials however, have been advocated in the literature and are in limited current use. For pH measurements, calibrating buffers containing added sodium chloride (saline buffers) have been described [3], as well as bicarbonate-based buffers [4]. Gases used for calibration of PCO_2 and PO_2 measurements are not of standard composition, but vary from laboratory to laboratory, especially with respect to oxygen; some laboratories even use a solution such as sodium sulphite [5] in lieu of an oxygen-free gas for zero calibration of PO_2. The calibrating gases themselves may not, depending on the method of formulation, contain the composition of gas that is stated on the accompanying label. Finally, use of tonometered liquids for calibration of PCO_2 and PO_2 measurements has been repeatedly reported [4,6,7] and is in routine use in a growing number of laboratories [3].

Why such diversity in calibration of instruments when basic design of the instrumentation is the same? The common reason in the literature seems to be: (1) a search for more reliable and accurate performance, and (2) a search for convenience. Modification of pH calibrating buffers has occurred for both reasons. Use of saline buffers coupled with use of a sodium chloride salt bridge is claimed to improve accuracy by reducing the sodium response of different pH electrodes, and improve reliability by reducing depletion of the salt bridge solution, as well as improving the stability of the liquid junction potential [3]. Use of the bicarbonate buffering system is more convenient, since one aqueous solution will serve as a pH, PCO_2 and PO_2 calibration point. Change in the manner of calibrating PCO_2 and PO_2 measurements has been attempted for the purpose of improving accuracy and reliability of these measurements. Differences in the PCO_2 and PO_2 readings between gases and liquids having identical gas tensions have been reported; for this reason, use of tonometered liquids having known values for PCO_2 and PO_2 has been investigated by others as an alternative to calibration using gases alone.

A major area of controversy in blood gas has been the question of whether changing the method of calibration will improve performance. PO_2 measurements have been singled out as being most in need of improvement, because of the tendency of the PO_2 sensor to read lower on liquid than on gas samples. Maas and coworkers [8] summarized the concern about the

[1] Figures in brackets indicate the literature references at the end of this paper.

accuracy of PO_2 measurements by presenting data concerning the average PO_2 readings obtained when blood samples having known PO_2 values were read on conventional blood gas instrumentation (table 1). The results showed a bias, the sign and magnitude of which were dependent

Table 1. Blood/gas difference in PO_2 measurement (after Maas, A. H. J. Mertens, P. J., *Clin. Chim. Acta*, 28, 443 (1970)).

O_2 (%)	PO_2 (mm)	PO_2 (mm)	Correction (%)
5	35	35.6	-1.7
10	70	71.1	-1.6
20	140	132.8	5.1
40	280	269	3.9
60	420	399	5.0
80	560	521	7.0

on the level of PO_2 in the sample being measured. Prior to Maas' studies, the bias was assumed to be a constant percentage of the sample PO_2; it was thought to be caused by the difference in diffusion coefficient in gas calibrant *vs.* liquid samples and, therefore, able to be corrected for in the final result. Maas' data, however, refuted this.

Data by Hulands and coworkers [9] showed another aspect of PO_2 performance that was equally disturbing (fig. 1). They showed that the magnitude of the PO_2 bias varied not

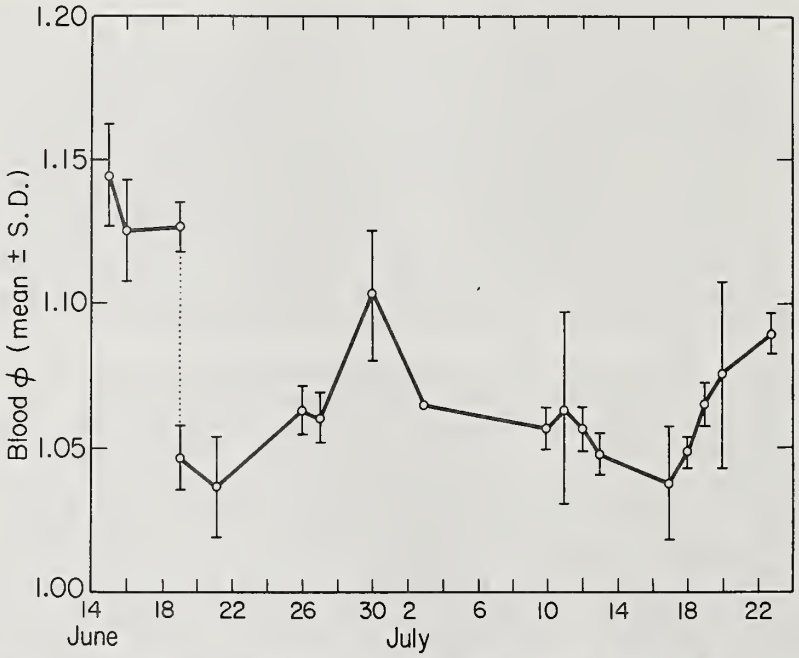

Figure 1. Day-to-day variation in PO_2 bias (from Hulands *et al.* [9]).

only according to sample PO_2, but also varied on a day-to-day basis that was unpredictable. Hulands felt that this was caused by the gas/liquid correction factor for the PO_2 electrode changing from day to day; he proposed to remedy this by calibrating the electrode directly with tonometered 30 percent glycerol solutions (fig. 2).

Our studies have centered on evaluating the clinical results obtained when gases vs. liquids are used for calibration of commercial blood gas analyzers. It seemed that one variable that was not able to be controlled in the studies of Maas and Hulands was that of sample preparation; blood tonometry and sample handling at high levels of oxygen tension is

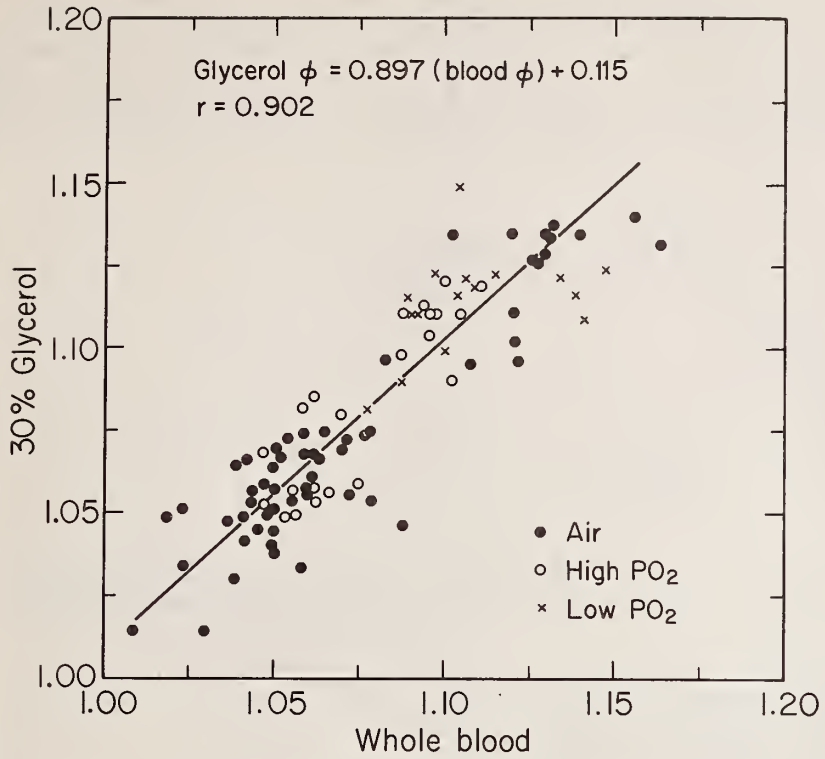

Figure 2. Paired measurements of 30 percent glycerol correction factor and whole blood correction factor (from Hulands *et al.* [9]).

difficult. Therefore, we decided to look directly at differences between instruments calibrated with gases and those calibrated with liquids. The two types of systems were set up side-by-side in the Blood Gas Laboratory at Peter Bent Brigham Hospital and used to measure blood gas values on patients' samples. The results, shown in figure 3, indicate that the variable bias described by both Maas and Hulands for PO_2 is not observed. Comparison data, where both instruments were calibrated with tonometered liquids, are shown in figure 4. The PCO_2 measurements that were observed in this study are shown in figure 5.

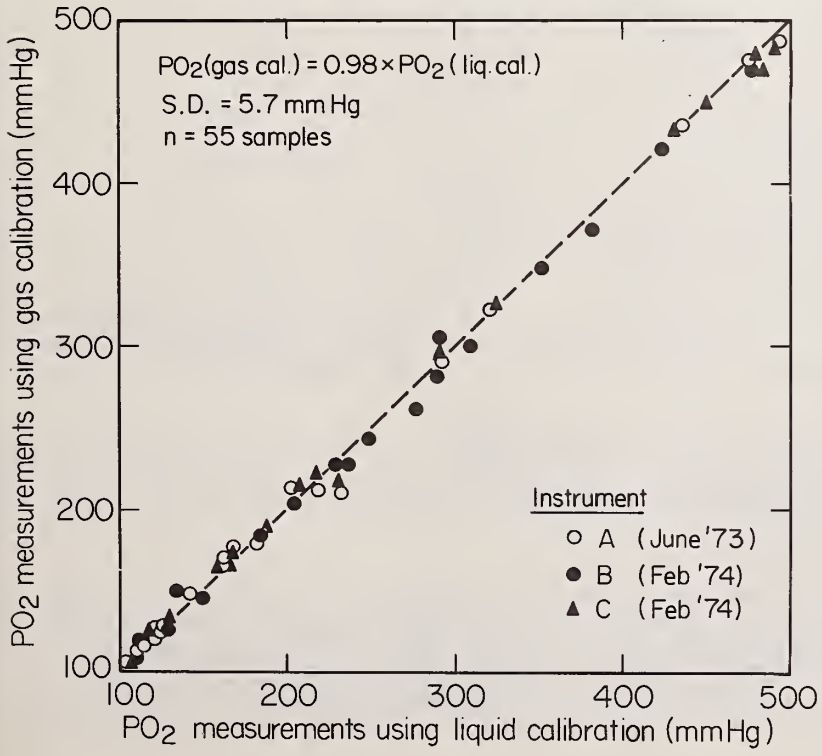

Figure 3. Comparison of gas *vs.* liquid calibration for blood PO_2 measurement.

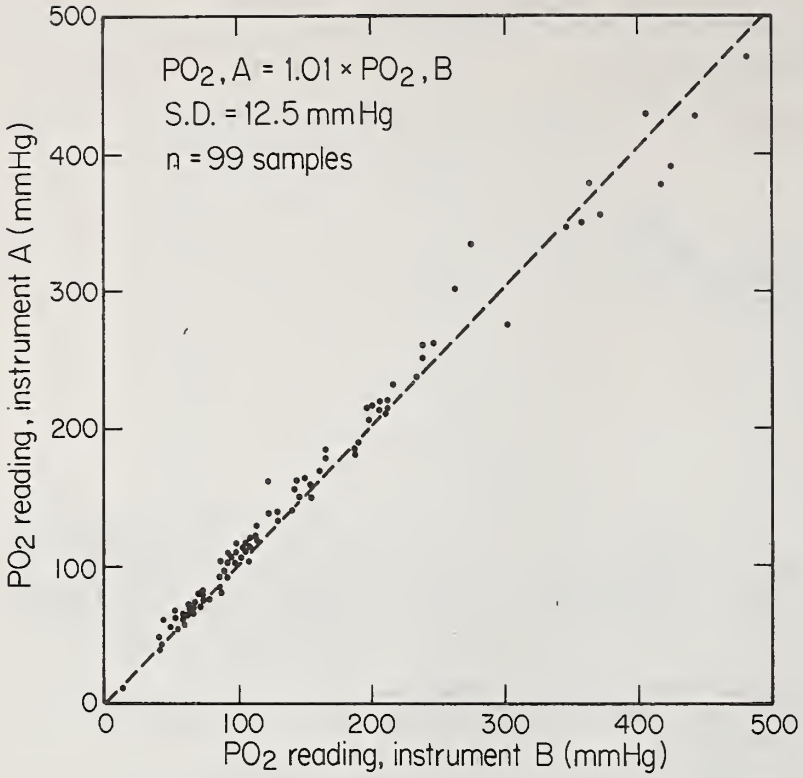

Figure 4. Comparison of liquid *vs.* liquid calibration for blood PO_2 measurement.

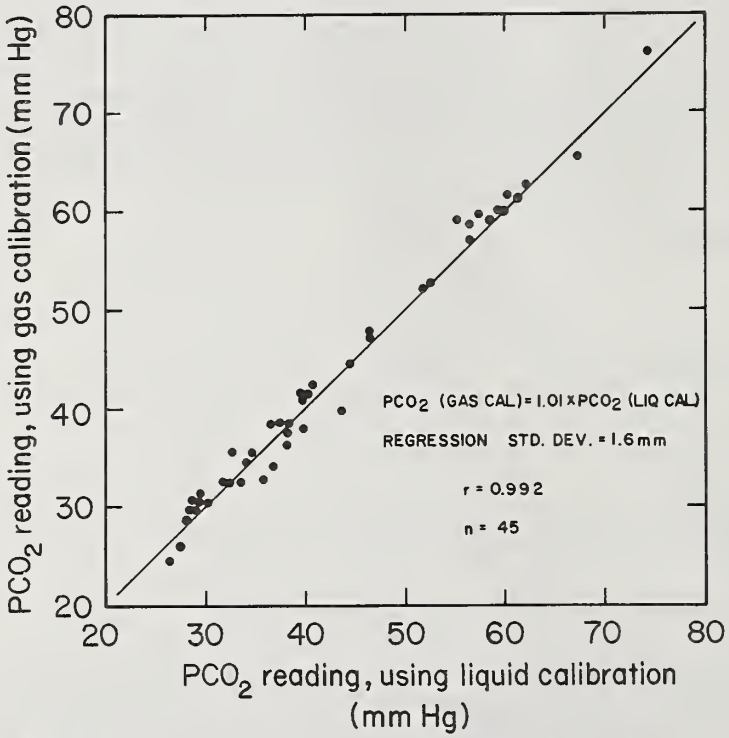

Figure 5. Comparison of gas *vs.* liquid calibration for blood PCO_2 measurement.

The data indicate that gas calibration of blood gas analyzers will yield PO_2 and PCO_2 values that are as accurate and reliable as those obtained when liquids are used for calibration. These results have been confirmed by more recent data in our laboratories on different types of equipment, and by Maas, who recently compared results obtained with instruments calibrated with gases and instruments calibrated with tonometered glycerol-water solutions [4].

It is intriguing to ponder why earlier data showed such a pronounced variation in results, both in terms of PO_2 dependent bias and in terms of a day-to-day variation in accuracy. We addressed this question by measuring the PO_2 of tonometered water solutions using a gas calibrated analyzer, and comparing these measurements with those obtained indirectly from oxygen content determinations on the same samples, using the Lex-O_2-Con oxygen analyzer. The results, shown in figure 6, are interesting: the oxygen content

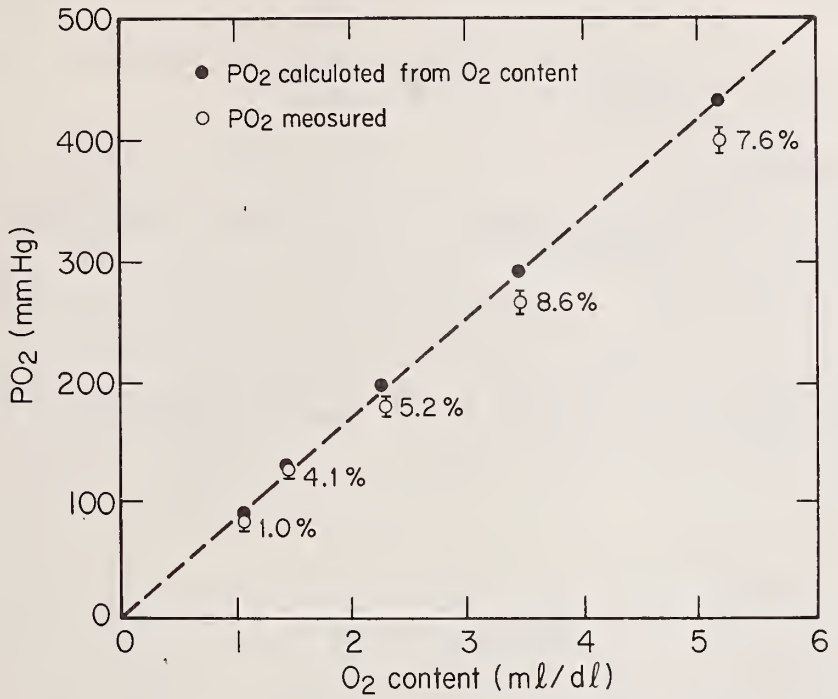

Figure 6. Comparison of measured PO_2 of tonometered water *vs.* PO_2 derived from oxygen content measurements on the same samples.

measurements on the samples at all PO_2 levels show that the tonometer performed as expected, with each sample giving the anticipated PO_2 value. However, PO_2 measurments on the same samples, using a blood gas analyzer, show the very type of PO_2-dependent bias as was reported by Maas. The conclusion from these data is that the variable bias reported by Maas is a function of atmospheric contamination, either during sample handling or instrument manipulation. Such contamination has been observed for every instrument studied and is manifested when recovery studies on tonometered blood or water are attempted. These conclusions should not imply that a gas/liquid correction for PO_2 measurements does not exist, but rather, that the correction factor remains a constant over a period of time. In our experience, the correction factor for present day blood gas analyzers is 5 percent, and varies no more than ± 1 percent. What is most important, is for manufacturers of blood gas instrumentation to explicitly state the accuracy of their instrumentation at all levels of PO_2 and PCO_2 so that users can effectively evaluate the performance of the total system without placing all the blame for poor recovery of PO_2 on a "high gas/liquid correction."

Calibration of blood gas instrumentation should be as simple and error free as possible, without compromising performance. Our studies, and those reported by Maas [4], indicate that gas calibration for PCO_2 and PO_2 measurements provides results which are comparable to those obtained when liquid calibration is used, when accuracy is used as the guideline.

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QUALITY CONTROL

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In my discussion of instrument specifications I indicated that better quality control (Q.C.) at individual sites was needed more than quality control of manufacturers. In fact, I can state categorically that without some type of on-site Q.C. program it is impossible to provide physicians with reliable blood gas measurements. Too many things can go wrong.

Attending physicians provide some degree of Q.C., but it is insufficient. If the data agree with what the attending physician expects, then he or she is satisfied. If the data provided by the blood gas instrument is not consistent with his or her expectations, then the physician does not believe the data. In this situation, some type of readily available referee sample is essential. Since blood gas measurements are made 24 hours a day, 7 days a week, the ideal Q.C. material would be available for immediate use at all times. Tonometered blood is, therefore, inadequate as an "instant" control for peripheral and central blood gas instruments around the clock. Tonometered blood, on the other hand, is the proper sample for use as a secondary standard.

More than 10 years ago, Dr. Arthur Babson of General Diagnostics developed a formula for a lyophilized serum control for acid-base assays. This lyophilized control is marketed as "Versatol Acid-Base." In 1970, when we first began our high volume blood gas service, I received many complaints from physicians during the first month of operation. At that time we did not use controls on every shift. The supervisor of the chemistry service, Ms. I. Fonseca, suggested that we run the lyophilized controls on every shift every day of the week. Once we began to utilize controls, at 3 different levels on every shift, the number of complaints fell to nearly zero. Now problems are discovered *in* the laboratory *before* results were reported. But the lyophilized controls are not practical for the distant units operated by non-laboratory personnel. Furthermore, the lyophilized controls lack assigned values for Po_2, although a single, but somewhat variable, Po_2 value for all 3 controls can be obtained by assay.

Following the introduction of our first peripheral blood gas unit it became obvious that a lyophilized control, or a whole blood tonometered control, was not adequate. What was required was an ampouled single-use control. Such a control has just been developed by Dr. James Turner and Dr. Arthur Babson of General Diagnostics. It is marketed as "Blood G.A.S. Control." Pilot lots of the ampouled controls have been available to investigators for more than a year. In fact, it was the availability of these unit-use controls for pH, Pco_2, and Po_2, at 3 different levels, that permitted us to expand our peripheral blood gas units in 1975.

The new ampouled controls have revolutionized our operation. We are able to leave a peripheral unit unattended and unvisited by any laboratory personnel for several days as long as ampouled controls are available at the peripheral site.

What happens when we open an ampoule? Table 1 shows that in 4 minutes there is little change in pH or Pco_2. The Po_2 data in this experiment cannot be interpreted since the Po_2 in the ampoule is so close to the Po_2 of room air.

How reproducible are the values for pH, Pco_2 and Po_2 when repeat measurements are made on 2 different instruments that are calibrated with the same gases and buffers? Table 2 shows the data for pH. The pH values are very reproducible. Table 3 shows the data for Pco_2. The Pco_2 is reproducible, but there is an obvious systematic difference between the two instruments even though they were calibrated with the same gases. Table 4 shows the data for Po_2. Po_2 is the least reproducible of the measurements at low values. This has to do with the particular instrument used. The IL 313 introduces a bolus of room air in front of

Table 1. Effect of exposure to air, mean of 6 ampoules in each of 3 different laboratories.

Time:	zero	30 s	60 s	240 s
pH	7.628	7.629	7.630	7.634
Pco_2	66.0	66.2	65.9	65.7
Po_2	163.5	163.8	164.5	163.2

Table 2. pH comparison of two IL 313's (same buffer).

Ampoule	No. 1	No. 2
1	7.422	7.419
2	7.421	7.416
3	7.419	7.420
4	7.417	7.419
5	7.417	7.424
Mean	7.419	7.420

Table 3. Pco_2 comparison of two IL 313's (same gas).

Ampoule	No. 1	No. 2
1	61	58
2	62	58
3	61	58
4	61	58
5	61	59
Mean	61.2	58.2

Table 4. Po_2 comparison of two IL 313's (same gas).

Ampoule	No. 1	No. 2
1	60	62
2	59	61
3	59	63
4	59	60
5	58	59
Mean	59	61

each sample. The controls do not have any buffering capacity for Po_2, therefore they are quite sensitive to the presence of oxygen in the instrument at any pressure other than the pressure in the control.

Are these controls useful when used routinely? Decidedly yes. Table 5 shows typical actual problems revealed by the control and not revealed by standard gas and buffer calibration.

Table 5. Typical problems revealed by controls.

Expected	Found	Problem
Pco_2 42	46	Needed new membrane
Pco_2 58	62	Needed slope adjustment
Po_2 100	80	Mold in wash solution
Po_2 100	Slow	Bubble behind membrane
Pco_2 58	Slow	Space between electrode and membrane too thick

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QUALITY CONTROL AND STANDARDS

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We have heard many different points of view on many aspects of blood gas measurements at this workshop. There is one point, however, upon which we all probably agree. We all feel that our own respective laboratories provide blood gas results which are of the highest quality available with modern technology. While this is undoubtedly true, if we were to survey our laboratories, we would find a wide range of methods for the basic standardization and quality control of these measurements in daily use.

This workshop should have several objectives in this area of standards and controls. First, we should strive to identify the methods and approaches which seem to be commonly agreed upon to produce accurate standardization and useful control. Secondly, for those areas where disagreement exists, we need to discuss the kind of experimental approaches which might resolve these questions. Lastly, we need to look into our own crystal ball and make some predictions on how standardization and control should be done in the future.

With these points in mind, I would like to express some of my own opinions on this subject and also describe some of my recent experiences with single phase control material.

Standardization should have optimally several characteristics. First, the material used should be precisely definable. By that, I mean it should be describable in terms of exact quantities of materials treated in a specific way to obtain the standard value. Second, the material should be as close in characteristics to the sample as possible. When dealing with whole blood as a sample, this is often difficult to achieve while maintaining definability. Lastly, the standardizing measurement should be done in the same manner as a sample measurement. Often the particular instrument used requires different sample application procedures for sample and standard.

Standardization of pH measurements meets these criteria reasonably well. Gas standardization, however, is another question. By far, the most widely used technique for gas standardization utilizes mixtures of pure gases flowing through a sample chamber. This technique violates two of the three basic principles of standardization. First, it is a gas while the sample is a liquid, and second, it is flowing, whereas the sample is static. The reason for its widespread utilization is that this material is easily handled and the resulting gas tensions are quite reproducible. As long as the flow rate of the gas is reasonably constant and humidification of the gas with water is accomplished at a constant temperature, good day-to-day reproducibility is attainable. Biases associated with the media and flow differences are accepted as the price of reproducibility. Thus, there is a gas-to-liquid correction factor associated with oxygen measurements which stems from the choice of a gas as the primary calibration medium [1][1]. Likewise, there is a difference in response characteristics of the primary sensors, such that the response time in the flowing gas is invariably shorter than the response time in a static liquid. Fortunately, both of these effects tend to be constant and day-to-day reproducibility is generally attainable. However, there is good evidence to suggest that during the operating life of these electrodes and prior to obvious malfunction, changes occur in these characteristics which are only evident by measuring liquids of known gas tensions.

[1]Figures in brackets indicate the literature references at the end of this paper.

Methods for correcting for these effects have included calculation of error factors, variation of sampling techniques [2], and equilibration of liquids with known gas compositions [3]. This latter effort was equally directed at defining a single phase control material. Sufficient progress has been made in this area to allow the recommendation of an all liquid system for pH and blood gas calibration [4,5]. Commercial equipment is now available to perform the equilibration in an accurate and simple manner so as to warrant utilization of these liquids as a standardization device.

The Radiometer Company has recently made a major advance in instrument design by incorporation of liquid calibration into their latest blood gas system. The ABL1 incorporates into its design a calibration system which utilizes various buffers equilibrated with mixtures of carbon dioxide and atmospheric air. It is important that we encourage Radiometer and other manufacturers to continue in this sound technical direction for future instrumentation. As modern equipment becomes more automatic, we are often required to use the calibration scheme chosen by the manufacturer even though better calibration methods might be available.

Moving on to the subject of quality control, I feel methods of control should have two additional characteristics over and above those mentioned already. First, they must be simple, because a tedious and complicated procedure just does not get utilized in a busy laboratory. And secondly, they should respond predictably to a developing malfunction before it becomes obvious without a control.

Many attempts have been made to prepare control systems with a whole blood matrix [6]. While these appear to have usefulness within a given laboratory, specimen variability and handling difficulties prevent universal application of tonometered whole blood. On the other hand, protein based controls for pH and PCO_2 are commercially available [7]. There has been a good deal of discussion as to the necessity of a protein based solution for quality control, the feeling being that a protein solution most clearly acts as whole blood would act. My attitude is that whatever advantages a protein solution might possess (and I am somewhat dubious that it has any), it is basically a nondefinable medium. I feel the reproducibility and predictability inherent in a tonometered pure aqueous solution makes it the media of choice for blood gas control.

Dr. Burnett and I reported on a particularly simple and also accurate device for the preparation of liquid controls [5]. Prior to the availability of this system, it was very difficult to get more than a subjective indication of the long term precision and accuracy of blood gas results. We have had now nearly two years experience with this approach. Table 1 shows cumulative daily statistics collected over a six month period at Hartford

Table 1. Blood gas quality control with tonometered bicarbonate (Hartford Hospital, 1974).

	pH (calc. val. = 7.35)		PCO_2 (calc. val. = 86 mm Hg)		PO_2 (calc. val. = 149 mm Hg)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Dec	7.34	.016	81	5	142	6
Jan	7.35	.012	79	4	145	3
Feb	7.34	.011	79	3	144	2
Mar	7.34	.022	84	7	146	3
April	7.36	.014	80	5	144	2
May	7.35	.012	83	4	145	3
Port Huron	7.35	.013	80	3	-	-

Hospital and similar data for a one month period at Port Huron Hospital. These data show the consistency of results obtainable over a long period of time with tonometered aqueous solutions. It also shows that interlaboratory comparison is also achievable with this material. Use of material such as this will allow meaningful comparisons of interlaboratory biases and errors. We have been in contact with several laboratories which have experienced initial difficulties in matching our expected values. After some consultation, we were able to identify serious unexpected system difficulties in some of the blood gas laboratories.

From the standpoint of commercially available materials, the General Diagnostics Company has developed a series of aqueous, gas-equilibrated liquids packaged in glass ampoules with assigned values of pH, PO_2, and PCO_2. This represents a particularly simple approach to avoid the complexity of tonometry. Table 2 shows some data which we obtained using this material in our laboratory. Notice that the precision obtainable with this material for pH and carbon dioxide is as good as that obtained with our own temperature-controlled tonometer. The oxygen results are somewhat influenced by variations in room temperature. However, a more important factor in the case of oxygen was that we had difficulties establishing a consistent method for transferring our specimens to a syringe for insertion into our instrument. It is expected that direct sampling will improve this precision. The real beauty of this product is that it makes blood gas control a very simple experimental process. Undoubtedly, this technological achievement indicates that we will soon see liquid standards available, packaged in a similar manner, perhaps even certified by NBS.

Table 2. Blood gas quality control with liquid-filled ampoules (Port Huron Hospital, February 1975).

	pH		PCO_2		PO_2	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
GD#1	7.06	.021	23	1.3	172	7.1
GD#2	7.39	.010	40	1.2	121	6.5
GD#3	7.64	.021	65	1.3	82	12.4

In closing my remarks here, I think we now have available the methods and materials to begin to resolve in an objective manner some of the nagging questions in blood gas technology. In our own laboratory we are beginning a study comparing the methods of quality control over a long period of time in regard to maintenance factors. I look forward to other activities directed toward the resolution of the problems in blood gas standardization and control.

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DEVELOPMENT OF REFERENCE METHODS: BLOOD GAS ANALYSIS

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1. Introduction

The nature of the parameters measured in blood gas analysis leads to unique problems in attempting to establish reference methods which will serve as an accepted norm against which manufacturers of instrumentation and practitioners of the method may compare the results which they obtain. The species are extremely labile both because of changes induced within the sample and also because of the ease of contamination of the sample by atmosphere. Metabolism of the sample results in lowering of P_{O_2} and elevation of P_{CO_2}, while mixing with air will reverse this situation.

Since the analysis of blood for pH, P_{CO_2} and P_{O_2} must be performed in an environment which may easily lead to errors resulting from contamination, reference methods must pay strict attention to both operator dependent factors and instrument dependent factors.

The primary purpose for establishing reference methods is to establish the precision and accuracy of the procedure used to obtain blood gas information in the clinical laboratory. As in any analysis, accuracy is defined as the expected- or absolute-value. The nature of the measuring system requires that the reference methods be established to define the accuracy and precision at the level where the P_{O_2} and/or P_{CO_2} values are being measured. It has been prevously documented that calibration for P_{O_2} should vary with the level measured [1,2][1]. Instruments calibrated to achieve P_{O_2} measurements which are within 2 percent of the expected value at 50 mm Hg must be calibrated differently to achieve the same performance at P_{O_2}'s of 300 mm Hg. The measurement of P_{CO_2} in the clinical laboratory is over a much narrower range, however, and this requirement is not evident for that measurement.

While accuracy of results is to be sought as a worthwhile goal, the importance of precision ought not to be underestimated. Monitoring of the clinical state of a patient while following the results of sequential measurements over several hours or days provides a clinically useful tool. Therefore, while results might even be in error in absolute terms, good precision of results will permit successful care of the patient.

[1]Figures in brackets indicate literature references at the end of this paper.

2. Operational Requirements

The number of factors which affect the accuracy of blood gas is significant. In order to organize them, they will be described as operator dependent factors and instrument dependent factors.

Operator dependent factors which affect results begin with the drawing of the blood sample for the analysis. There will be no attempt to deal with the actual technique used by the clinician in drawing either arterial or venous samples. The attempt will be made, however, to delineate aspects which can affect blood gas results by actions taken both before and after the sample is drawn.

The syringe used to draw the blood will have an effect on the results achieved. There is literature which indicates that only glass syringes permit accurate results [3] while other literature indicates that disposable plastic syringes are suitable [4]. Our experience has been that as long as the blood is not stored for extended periods between drawing and analysis, no significant bias is introduced. If samples are stored under iced conditions for several hours, changes consistent with increases in P_{O_2} and decreases in P_{CO_2} caused by diffusion through the walls of the plastic syringe are observed when samples stored in plastic are compared with samples stored in glass.

The amount and nature of the anticoagulant used also affects the blood gas results achieved for pH and P_{CO_2}. The anticoagulant of choice for blood gas analysis is sodium heparin [5]. At the present time, however, standardization of the number of units of heparin per ml of sample is not adequately defined. As a result, variation in the volume of anticoagulant used affects results through dilution of the sample, as well as through pH changes induced by using other anticoagulants such as ammonium heparin [6].

For the measurement of P_{O_2} and for the direct photometric measurement of oxygen saturation, the choice of anticoagulant is not so critical since the only effect on these measurements would be through dilution of the sample by the oxygen contained in the anticoagulant.

Once the operator has a properly prepared sample, the next factor under the control of the operator is the proper calibration of the blood gas analyzer. Accuracy of calibration of P_{O_2} and P_{CO_2} is dependent on the accuracy of the calibrating media, whether liquid or gas, and on correction for barometric pressure changes. Manufacturers of calibrating gases standardize their values through either the Scholander technique or gas chromatography. Within a given geographic region, day-to-day fluctuations in barometric pressure will be small, but many, if not most locations will have a standard barometric pressure lower than the 760 mm Hg standard at sea level. At 3300 feet elevation the barometric pressure would average about 674 mm Hg.

The accuracy of blood gas results, even when each of the necessary calibration steps is carried out properly, is also dependent on the operator. The instrument must be given proper maintenance. At present, totally blood compatible materials are not available for construction of blood gas analyzers. Therefore, regular routine cleaning of the system is necessary to prevent buildup of protein residues from previous analyses. The advent of automation in blood gas analysis insures that samples will each be handled similarly once they are inducted into the instrument, but without proper maintenance, even these systems will not yield results which are accurate in the long term.

Instrument dependent factors present in achieving accurate, precise results in blood gas analysis deal primarily with the proper calibration of the sensors and introduction of the blood sample into the measuring chamber of the system.

In the near term, establishment of standards for blood gas calibrators is required. At the present time, the use of phosphate buffers serves to establish the calibration of the glass electrode for monitoring pH of blood [7]. Calibration of the gas sensors, however, continues to be a source of some controversy. The use of standardized, humidified calibrating gases flowing past the tip of the sensors has been the calibration method of choice for many systems. However, the question of the gas/liquid membrane correction factor [8] for oxygen measurements has yet to be adequately resolved. Proposals have been made for alternatives such as tonometered glycerol-water solutions containing phosphate, bicarbonate and chloride [9] to serve as the calibration medium. Proposals for other fluids such as tonometered blood [10] and sodium bicarbonate--sodium chloride solutions equilibrated with known gas mixtures [11] have also been made, the latter being proposed as a quality control procedure.

3. Methodology Currently Employed

Currently available control materials for characterizing the reliability of blood gas results fall into two categories. The first category includes serum-based controls that are reconstituted by addition of an appropriate solvent. The resulting solution, once reconstituted, is then inducted into a calibrated blood gas analyzer and the results are compared with the nominal values to be expected. These require little time to reconstitute and are relatively simple to use. Disadvantages in attempting to follow this approach, however, to develop a standard reference material include the requirement for a high degree of care in mixing with the solvent to prevent undue contamination with air before sampling, the fact that the values are established by the same technique as the method being tested, the temperature coefficient is different from that for blood, and the material must be handled differently than a blood sample would be handled. This last would lead to incorrect assumption either of problems or lack of them.

Another control material which might be considered is the aqueous based control material which is supplied in liquid form with known values of pH, P_{CO_2} and P_{O_2}. In this case the mixing step is completely eliminated. The solution need only be inducted into the blood gas analyzer and the results recorded. The disadvantages here, however, are similar to the serum-based material above with respect to a standard reference material. First of all, it

is simply an aqueous solution, not blood. Also, the range of values available is limited of necessity and may not include some of the more extreme ranges where blood gas analysis may be performed.

In addition, neither of the above approaches tests the operator's ability to properly handle blood samples under controlled conditions. As noted previously, this is an important factor in achieving accurate blood gas results.

4. Tonometry of Whole Blood

It is proposed that the method of choice for developing a reference method for blood gas analysis be the tonometry of whole blood. This approach has a number of advantages. First, the method being used is the primary method. Second, all controlled parameters are independent of the measuring system, *i.e.* established without reference to a blood gas analyzer. Equilibration of the whole blood can be made with gases having accurately known P_{O_2}'s and P_{CO_2}'s. Instrument response will be identical to that when clinical samples are being measured. The operator is handling whole blood in the same way as a clinical sample once the blood is withdrawn from the tonometer. Any deviations observed will be identical to those to be experienced with clinical samples. Temperature coefficients are identical, for example. The pH of whole blood may be adjusted by addition of bicarbonate so that it is both known and predictable under specific tonometry conditions to be used to establish the P_{O_2} and P_{CO_2} values desired.

Below is shown the results achieved using the approach described. A volume of whole blood was standardized to a known pH by addition of bicarbonate. This was then sealed into containers and kept refrigerated. Over a thirty-day period these blood samples were tonometered with gases of known composition, and the pH, P_{CO_2} and P_{O_2} determined after tonometry. The results are tabulated as shown. The analyses were performed both at Instrumentation Laboratory, as well as at another institution. The gases used for tonometry were:

	Location No. 1		Location No. 2	
Gas No.		SD		SD
1[a]	pH: 7.370	0.006	pH: 7.360	0.007
	P_{CO_2}: 49.5 mm Hg	0.6	P_{CO_2}: 46.5 mm Hg	2.0
	P_{O_2}: 51.7 mm Hg	1.0	P_{O_2}: 51.4 mm Hg	1.95
2[b]	pH: 7.429	0.007	pH: 7.420	0.010
	P_{CO_2}: 35.8 mm Hg	0.44	P_{CO_2}: 33.9 mm Hg	0.77
	P_{O_2}: 139.4 mm Hg	1.4	P_{O_2}: 137.5 mm Hg	2.1
3[c]	pH: 7.511	0.006	pH: 7.500	0.013
	P_{CO_2}: 21.7 mm Hg	0.4	P_{CO_2}: 20.7 mm Hg	0.58
	P_{O_2}: 75. 1 mm Hg	1.3	P_{O_2}: 75.00 mm Hg	2.8

[a]Gas No. 1: 7.0 percent O_2, 7.0 percent CO_2, balance N_2

[b]Gas No. 2: 20.0 percent O_2, 5.0 percent CO_2, balance N_2

[c]Gas No. 3: 10.0 percent O_2, 3.0 percent CO_2, balance N_2

Thus, it can be seen that consistent results may be achieved over a period of time using tonometry with gases of known composition to bring whole blood samples into gas equilibrium. Although the range shown here is relatively limited, it could easily be extended to, for instance, high P_{O_2}'s by use of a gas having a P_{O_2} of 300 or 400 mm.

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QUALITY CONTROL AND STANDARDS

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The term "quality control" can be interpreted in various ways. In a narrower sense, it can thus be taken to mean no more than a quality check-up of a measuring system. In a wider sense however, it aims at establishing whether the results of tests performed on a sample actually do represent the bodily condition of a patient. It is, of course, this latter objective of quality control that is the ideal one, but unfortunately it is not always attainable.

The flow diagram for a sample is seen in figure 1. A proper quality control is performed by letting a known sample run through the whole system--in other words, it is the reference person we are looking for. It has been attempted [1][1] as a tool for controlling the "daily mean" to take blood samples from the staff every day and then use the results as control material. It is quite natural to use blood in quality control as it is the sample material, but blood is a very special liquid and it is difficult to imitate the physical and chemical behavior of blood in other media. When trying to simulate blood, it is necessary to get the correct buffer capacity for pH, Pco_2, and Po_2, where the oxygen buffer is the most difficult to establish. It is necessary to simulate what is called the suspension effect from the red blood cells when measuring pH. It is necessary to simulate dissolved protein and the viscosity of the blood. A list of specifications can thus be made, from which a lot of practical "artificial blood references" can be produced.

Figure 1. Flow diagram for running a sample.

In some types of analysis, it is possible to get very close to the ideal condition, *e.g.*, serum reference materials used in quality control of the inorganic salts are excellent synthetic substitutes for the genuine sample. But as far as pH and blood gases are concerned, it is not possible to produce the stable reference material that is a necessary condition for carrying out quality control [2].

Quality control is performed as spot tests, but no test can improve a weak system. The spot tests are therefore unable to solve quality problems; they can only indicate that something is going wrong. This means that one cannot merely concentrate on finding the best of all reference materials. It is equally important to ensure that inbuilt security in the system is provided. Internationally accepted rules for the application of reference methods, units, formulas for derived parameters, choice of derived parameters and sample handling are some of the indispensable conditions on which the quality control must be based. Internal quality control is much to be the preferred, but technical limitations, *e.g.*, stability problems, and legislation requirements are some of the reasons why external quality control is utilized. Western European countries, and especially the Medical Society

[1]Figures in brackets indicate the literature references at the end of this paper.

of West Germany have shown a great interest in external quality control. Their guidelines do not deal with blood gases, however, as they have based their quality control on test sera. External control may be a useful addition to laboratory control, but the time delay involved is reason enough for rendering it unsuitable as a primary control basis in blood gas analyses. As a first step to quality control of blood gases, collaborative control within small geographical regions is a possibility. This could be a valuable, although expensive, way to build up a control system.

This author feels that it is important to establish that both choice of derived parameters and units are part of a quality control program. One of the most important things in quality control is the log book. Without recording the trends and the change in scatter of the results, it is impossible to foresee the coming break-down of the system, which always occurs at the most inconvenient time in the day. To reduce the shut-down period, the quality control log book is thus an indispensable tool.

It is very difficult to solve the entire quality control problem for pH and blood gases, but even a partial solution will be a great improvement over the present situation. Before starting a control program, it is natural to ask what limits of accuracy and precision are necessary to give the control a meaning. Manufacturers usually refer to the instrumentation specifications. However, these specifications are a result of technical limitations and, therefore, are not necessarily in agreement with the clinical requirements. The best plan is to let clinical needs, not the technical ability, determine the limits of accuracy and precision. This point is very important.

Some authors [3] are of the opinion that the methodological inaccuracy must not be greater than one SD of the normal range. The normal range is then 4 SD, which incorporates both the biological and the methodological variation. For the blood pH, this means an accuracy around 15 mpH, which should be obtainable. In Pco_2 measurements, the same range is 3.5 mm Hg, which can be easily reached.

As regards the separate parameters, pH is the best established. There is a definition of pH--whether good or bad--and there are buffers which can be used to calibrate the electrode. Pco_2 can be determined directly with a gas-sensing electrode, or it can be determined indirectly by means of the equilibration function of the sample. In either case, an analyzed gas mixture is the basis for evaluating the quality of the measurement. Po_2 is determined directly with a Po_2 electrode, and here again gas mixtures form the basis of calibrations.

The lack of a reference material is not the only hindrance to careful control. Blood-gas measurements are typical stat measurements of one to two samples at a time, which means a Q.C. every second sample is desirable, and that is, in practice, impossible. A complete calibration of the system is therefore preferable just before the measurement is performed, but in practice even this is normally not possible. A compromise involving frequent calibrations during the day can, however, improve the quality of the measurement.

For calibrating purposes, gases are satisfactory, but as a medium in quality control, a liquid is much to be preferred. A minimum requirement is that quality control shall be performed on a liquid with known partial pressures of carbon dioxide and oxygen--in other words--a tonometered test solution. Blood is, of course, the preferred liquid for that purpose. The preferable control procedures are listed in figure 2.

pH: Buffer, Ton. bicarbonate solution

PCO_2: Blood, Ton. bicarbonate solution

PO_2: Blood, (Ton. aqueous solution)

Figure 2. Liquids for quality control.

It is not a sophisticated way of performing quality control, but, on the other hand, many laboratories do not even reach this level, and those that do are satisfied. That leads to another problem, namely the gap in the technical standard between the leading laboratories

and the ordinary ones, even in relatively homogeneous countries. It is very important that this gap in technical standard does not grow even wider, thereby creating a situation where quality control procedures become unattainable for lesser laboratories so that there is a risk that they will refrain from using them. Quite likely, such laboratories would be the ones that stand in greatest need of quality control.

The development problem has other aspects. If the laboratories are unable to prove their willingness to document the quality of an analysis, some other authority will take the initiative and create rules which, in the worst case, are impracticable, and in the best case, impractical. The same demands must be made with respect to the industry that produces equipment and diagnostic products for the laboratory, as a quality control program will only be a success if complete confidence exists between authorities, laboratories, and the industry involved.

The tonometer technique will no doubt give a lot of problems owing to inadequate training and experience. The only possibility is to promote the tonometer technique and then to learn from experience whether it is usable for control purposes. The manufacturers of blood-gas equipment must therefore be prepared to take the great responsibility for producing tonometers of a reliable and simple technical construction. Today, only a few companies produce a commercial tonometer. A tonometer, however, is only the tool used to produce gas equilibrium. The standard is the gas mixture which is used to equilibrate the blood.

Gas mixtures can be obtained everywhere but they have to be analyzed before they are usable as standards in tonometry. That is a point where cooperation on an international plane could produce fast results. Determination of the compositions of gases is purely a measuring problem, but internationally certified gas mixtures could remove one uncertainty from tonometry and also from the calibration procedure.

From a technical point of view, tonometered buffers are easier to handle than blood, and it might be worthwhile to try to evaluate in practice all the differences that can occur between blood and aqueous solutions. This compromise using buffers instead of blood, can give the unskilled laboratory a chance to start a control program without being completely lost from the beginning. Because blood-gas analyses are so complicated, the need for good quality control is immense, but I believe that progress here is more a matter of perspiration than of inspiration.

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DEVELOPMENT OF REFERENCE METHODS

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The precision of a measurement can be determined without having a reference method, whereas the accuracy may elude the determination without an established and well-documented reference method. The reference method is the most reliable method irrespective of analysis speed and cost, which implies that the reference method is not necessarily identical with the routine method.

A routine analysis is the practical procedure adopted by a particular laboratory. A routine analysis is, of course, tested against the reference method to expose the limitations of the former. The routine method, which may not be the reference method, is classified in the group of methods containing bias--known or unknown. It allows one to select just that method of analysis that meets the prevailing conditions (*viz.*, local legislation, technical training, instrumentation, and future development).

A proposal for categorizing analytical principles has been made by IFCC. According to this definition, a reference method is a method with an accuracy = $0 \pm \delta$, where δ is negligible in comparison with interlaboratory imprecision. The ultimate method, called "definitive method", has no known source of inaccuracy.

In order to obtain the total concept, a definitive method must be available. For pH it is not possible to set up a definitive method, as I will explain later, but for both Pco_2 and Po_2 it is--at least in theory--possible to measure the partial pressure in a system by known, established techniques. What I have in mind is to try to investigate a method for measuring the total amount of carbon dioxide or oxygen in an infinitely small quantity of gas. The problem with blood is that the total content of carbon dioxide or oxygen does not give any information on the partial pressure of the gases in the blood. In other media it is, however, possible to get such information due to simple dissolving processes in these media. By equilibrating, *e.g.*, a small piece of plastic in the blood and measuring the total gas content, the partial pressure can be calculated. The only limitation to such a method is the technical problems. With the advanced technical skill of today, it should, however, be possible to develop a "definitive method" based on this principle, but as far as I know this has not yet been attempted in practice.

The very restrictive definition of reference methods is difficult to meet especially for such complex systems as pH and blood gases. The day when such reference methods are available lies somewhere out in the future, and in the light of this definition even pH is a doubtful parameter. pH determination on blood is based on an operational definition, using a hydrogen electrode and a saturated KCl liquid-junction. Especially the residual liquid-junction potential gives a more or less unknown bias on the measurement. Nevertheless, no one will deny that pH in blood is a valuable and important parameter, and there are, in fact, no means of eliminating the liquid-junction problem as long as electrochemical cells are used to measure the hydrogen ion activity. The normal way of measuring pH is, of course, to use a glass electrode instead of a hydrogen electrode. The junction potential can be changed by using other types of reference electrode systems, but it cannot be eliminated and it is unlikely that anyone has tried to measure blood pH with a hydrogen electrode! What can be done is to accept the pH definition as it is or, better still, to improve it with a more precise description of the liquid-junction type of salt bridge and the geometry of the junction.

pH is a good example of a practically defined parameter which has shown its worth, even though several standardizing committees have tried to change the units for pH. It is my opinion that we still have the best of all proposed solutions to pH in the operational pH definition, which requires only small changes to lead to what we may call an "assigned method."

Turning to the Pco_2, matters are far more complicated. It may be possible, as already mentioned, to think out a definitive method which, no doubt, would be useless in practice. There are two established methods for determining Pco_2, one measures the CO_2 partial pressure with the Severinghaus-type carbon dioxide electrode. The other is the equilibration method, known as the Astrup technique, developed by Astrup and Siggaard-Andersen. Neither of these techniques is a direct measurement of CO_2 pressure or carbon dioxide activity. It may seem obvious to favor the electrode technique, as one can argue that an electrode gives a more direct measurement than the equilibration technique.

As all other reference methods, the CO_2 reference methods require that certain conditions are defined, for example, fixed and known temperature, oxygen saturation of the sample and hemoglobin concentration. However, a lot of problems connected with the electrode are known, and can be classified as interferences. Leakage of hydroxide ions from the pH glass surface, different osmotic pressure in the sample and the electrode electrolyte, and loss of gas into the bulk electrolyte, are some of the problems which are not easily overcome.

Using the Astrup technique as reference method is not the ideal solution, but for several reasons I feel that this method offers certain advantages. By tonometering the same sample as used for measurement, the equilibration curve is established directly from the sample. The tonometry process will offer a fixed saturation. When using an electrode, Pco_2 is converted to pH in an electrolyte layer after diffusion across a thin plastic membrane, and a calculation is necessary. In the equilibration technique, on the other hand, the electrode--indeed, one of the most reliable ones--is used for both calibration and measurement. The Astrup method thus eliminates the Henderson-Hasselbalch equation. One disadvantage of the equilibration is the need for defined or known oxygen saturation of the blood sample. The Astrup technique will, of course, not oust the gas electrode technique, but it is my opinion that we have a better theoretical background when using an equilibration technique as reference method.

The Po_2 analysis is, no doubt, the most complicated one for which to propose a reference method. Compared with Pco_2, for which two well-established methods are available, Po_2 offers only one method, namely the Po_2 electrode itself. Admittedly, an electrode which in practice has shown its validity, but whose theoretical basis is very weak, has a lot of known interferences and side-reactions. To found a reference method on the Po_2 electrode itself will hardly lead to success.

Here, too, there is the possibility of using an equilibration technique. If one has a precise knowledge of the oxygen dissociation curve, then the electrode technique can be converted into an optical determination of the oxygen saturation. Experience with the optical saturation measurements are fairly good, but the other side of the problem--prior knowledge of the oxygen dissociation curve--may kill the idea. Several parameters change the dissociation curve. Examples are pH, Pco_2, and enzymes (2,3-DPG). I feel that optical analysis based on a carefully selected set of definitions in order to lock the dissociation curve in position would provide a better reference method than any system founded on the polarographic oxygen electrode. If the outlined methods are discovered to be useless, only the definitive methods are left. As a matter of form, it may be recalled that potentiometrically working oxygen electrodes based on redox-potential measurements do exist, but so far, problems connected with the irreversibility of the material have limited its use. Someday, a good, reversible, oxygen-binding substance may lead to workable electrodes.

pH	none
P_{CO_2}	determination in "microbubble"
P_{O_2}	determination in "microbubble"

Figure 1. Definitive methods.

pH	glass electrode
P_{CO_2}	Astrup method
P_{O_2}	saturation equilibration technique

Figure 2. Reference methods.

The reason for choosing a method based on equilibration both for carbon dioxide and for oxygen is that, from a scientific point of view, the processes are better known. From a practical point of view, the suggested methods are more debatable, and a simpler way to solve the reference method problem is not to base the measurements on the gas sensing system, but to use the tonometer as the reference method. That means to develop a method which is able to produce a reference material--the tonometered blood sample.

I have now tried to establish a system of methods which could serve as an inspiration in our endeavors to develop a suitable reference method. No doubt, it will be clear to everybody that, so far, these methods are carried out only as suppositions, but they do at least expose the fields which in my opinion ought to be studied.

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STANDARDIZATION OF ION-SELECTIVE ELECTRODES FOR SERUM ANALYSIS

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Electromotive-force measurements at 37 °C in synthetic electrolyte mixtures simulating serum provide evidence that ion-selective electrodes respond in a near-Nerstian manner to changes in concentrations of the species to which they are selective. The relatively minor effects of changes in ionic strength on the electrode potentials are almost entirely accounted for by changes in the activity coefficients. It is suggested that similar electrolyte mixtures may serve as calibrating standards in clinical monitoring with ion-selective electrodes. The lanthanum fluoride electrode shows promise as a useful reference electrode in media of this sort.

1. Introduction

In the decade which has passed since their introduction, ion-selective electrodes have found increasing use in a variety of biological applications [1,2].[1] In addition to electrodes designed to measure electrolytes in body fluids, novel devices based on the "building block" principle [3] have been constructed to determine other clinically important entities such as urea [4] and glucose [5]. Changes in the configuration of existing electrodes, such as immobilizing the exchanger in a polymer matrix, have resulted in highly reliable and rugged devices [6].

In the analysis of electrolytes, an important advantage of ion-selective electrodes over existing clinical methods such as photometry is that they respond to *free* ionic activities or concentrations rather than to total concentrations. If one could obtain free ionic concentrations (which are the medically significant quantities) from measured ion-selective electrode potentials, the utility of ion-selective electrodes would be very significantly enhanced. Such a procedure is dependent upon the development of reliable calibration procedures, particularly in the complex mixtures of electrolytes found in biological fluids. Two major problems must be faced in establishing suitable standards. First is the experimental limitation imposed by the imperfect selectivity of the electrode; second is the theoretical difficulty attending a conversion of activity to concentration.

2. Selectivity

The empirical Nicolsky-Eisenman equation [7] is useful in describing the response of an electrode selective for an ion in the presence of an interferent j:

$$E_{el} = \text{Constant} \pm \frac{RT \ln 10}{z_i F} \log \left[a_i + \sum_n k_{ij} a_j^{z_i/z_j} \right] \qquad (1)$$

where E_{el} is the electrode potential, a_i and a_j are the activities of the primary ion and the interferent, respectively, z_i and z_j are the corresponding electrical charges, and k_{ij}

[1] Figures in brackets indicate the literature references at the end of this paper.

is the selectivity ratio. This equation holds fairly well at low interference levels but, generally speaking, the selectivity ratio is not a constant and depends on the activities of i and j. Since the relative concentrations of ionic constituents in body fluids are likely to vary under disease conditions, it becomes necessary to examine the extent to which measured potential differences are affected by selectivity parameters.

3. Activities *vs* Concentrations

The development of an operational pH scale was facilitated by the availability of buffer substances spanning a wide range of pH values at comparatively low ionic strengths between 0 and 0.1. This not only enables one to separate conventionally the thermodynamically measurable mean ionic activity coefficients into single ion activity coefficients but assures minimal residual liquid-junction effects in practical measurements. In the calibration of ion-selective electrodes, used to determine the clinically important ions, the ionic strengths of the solutions may exceed 0.1 and may vary rather widely, spanning a wide range of activities [8]. This would mean not only that the simplest formulas for the conventional separation of mean ionic coefficients into individual ionic contributions would no longer be valid but that the residual liquid-junction potentials might be large enough to cause appreciable errors in the measurements.

Metal-ion buffers offer a possible solution. Although buffers for calcium ion, based on the EDTA and NTA complexes, have been proposed [9], the measured pCa values depend heavily on the accuracy of the conditional formation constants. Furthermore, ligands complexing with Na^+, K^+, and other alkali metal ions of sufficient stability in aqueous media are not available.

Despite the success of the convention based on hydration numbers [10] in certain special cases, the evaluation of single ion activity coefficients in multicomponent electrolyte mixtures continues to pose considerable difficulties. On the other hand, the ionic strengths of body fluids are sufficiently low (usually less than 0.17 mol l^{-1}) that the actual single ionic activity coefficients are likely to be reasonably close to the values derived from the conventions that have proved useful at ionic strengths less than 0.1. Taking these factors into consideration, suitable standards for calibrating ion-selective electrodes for use in biological fluids are provided by a set of synthetic electrolyte mixtures whose ionic concentrations span the range normally encountered in clinical analysis. It has been shown [11] that the electrode potential responds in a near-Nernstian manner to changes in the *concentration* of the ions of interest at the normal ionic strength of serum, although the slopes $S = E_{el}/\log C$ may depart slightly from the values given by theory. In addition, it appears possible to apply corrections for changes in ionic strength if these occur due to disease conditions.

4. Emf Measurements in Standards of Constant Ionic Strength

Measurement of electromotive force at 37 °C in cells with and without liquid junction were made with a series of calibration standards in which the concentrations of Na^+, K^+, Ca^{2+}, H^+, and Cl^- were varied simultaneously between the lower limits encountered under clinical conditions and their normal values. The indicator electrodes used in the study were: sodium glass electrode (Corning), potassium electrode (valinomycin exchanger in diphenyl ether), calcium electrode (Simon lipophilic Ca^{2+} exchanger [12] immobilized in polyvinyl chloride), and pH glass electrode (Corning). Tris(hydroxymethy)aminomethane ("Tris") was used as the buffer substance. The buffer ratios in the reference solutions were varied and pH derived from $pK'_{BH+} = 7.907$ in isotonic saline at 37 °C; this apparent dissociation constant for protonated Tris was calculated from data given by Durst and Staples [13]. Nitric acid and sodium nitrate were used to vary the buffer ratio and to maintain the ionic strength constant at 0.16 mol l^{-1}. Details of the preparation of the standard solutions and other experimental procedures have been given elsewhere [11].

Compositions of the standards of constant ionic strength are given in table 1. As already mentioned, it is important to eliminate or minimize liquid-junction potentials in order that the measured potentials truly reflect changes in ionic activities or concentrations. For this reason, particular attention was given to the use of cells without liquid junction, with reference electrodes immersed directly in the standard solutions. The silver-silver

Table 1. Compositions of calibration standards.[a] (All solutions have an ionic strength of 0.16 and contain 1 mmol NaF per liter.)

Standard Number	C_{Na}	C_K	C_{Ca}	C_{Cl}	pH
1	100	1.0	0.30	66.6	7.90
2	105	1.4	0.38	70.0	7.80
3	110	1.8	0.46	75.0	7.70
4	115	2.2	0.54	80.0	7.60
5	120	2.6	0.62	85.0	7.55
6	125	3.0	0.70	90.0	7.50
7	130	3.4	0.78	95.0	7.45
8	135	3.8	0.90	100.0	7.40
9	140	4.0	1.00	106.0	7.36

[a] C_i in mmol l^{-1}

chloride electrode is of limited usefulness here, since the chloride ion is one of the constituents being monitored. The reliability of the lanthanum fluoride electrode as a reference was investigated. Accordingly, small concentrations of fluoride ion were added to each of the standard solutions. Possible side reactions such as precipitation of calicum fluoride or adsorption of the fluoride ion on the walls of the containing vessel were shown to be of little concern [11]. The emf of three types of cell, namely

SCE || Bridge Solution || Standard | ISE A

Ag;AgCl | Standard | ISE B

and

LaF_3 | Standard | ISE C

was measured. Steady potentials (to ±0.1 mV) were usually obtained after about 45 minutes.

In order to obtain the indicator electrode slopes from data for cells of type B, the contribution to the total cell emf made by the changing chloride ion concentration was allowed for by assuming Nernstian response of the Ag;AgCl electrode. It is evident from the least squares regression parameters for different electrode pairs given in table 2 that the electrodes responded in a near-Nernstian manner to changes in the concentrations of the ions being sensed when the ionic strength was constant.

This conclusion can be confirmed by examining the variation of the activity coefficients in individual cases. For example, the emf of cells of type B can be written as

$$E = E^{\circ\prime} + S \log C_M y_+ + 61.54 \log C_{Cl} y_- \qquad (2)$$

where C_M and C_{Cl} are the concentrations of the cation being sensed and the chloride ion, respectively, and y_+ and y_- are the corresponding activity coefficients on the molar scale; S and 61.54 are the Nernst slopes (in mV) at 37 °C for the indicator electrode and the Ag;AgCl electrode, respectively. On rearrangement, we obtain

$$E^{\circ\prime\prime} \equiv E^{\circ\prime} + S \log y_+ + 61.54 \log y_- \qquad (3)$$

$$= E - S \log C_M - 61.54 \log C_{Cl} .$$

Table 2. Parameters for the calibration of ion-selective electrodes in standards of ionic strength = 0.16.

Indicated ion (i)	Reference electrode	E_{cell} (mV at 37 °C)	S.D.[a]
H^+	SCE	435.1 - 60.9 pH	0.15
	LaF_3	437.0 - 61.15 pH	0.32
	Ag;AgCl	0.68 - 62.20 pH	0.11
K^+	SCE	63.3 + 57.3 log C_K	0.16
	LaF_3	58.14 + 55.4 log C_K	0.40
	Ag;AgCl	0.38 + 58.9 log C_K	0.16
Na^+	SCE	36.58 + 54.04 log C_{Na}	0.06
	LaF_3	37.06 + 54.9 log C_{Na}	0.27
	Ag;AgCl	0.35 + 56.4 log C_{Na}	0.21
Ca^{2+}	SCE	124.9 + 27.8 log C_{Ca}	0.10
	LaF_3	122.9 + 27.3 log C_{Ca}	0.29
	Ag;AgCl	0.25 + 29.8 log C_{Ca}	0.14

[a]Standard deviation for regression, in mV.

Constancy of the right-hand side of eq. (3) will confirm the invariance of the activity coefficients. From the low standard deviations for the values of E°" (table 3), it is evident that the activity coefficients remain substantially constant throughout the range of calibration.

Table 3. Constancy of activity coefficients in calibration standards of ionic strength = 0.16.

Indicated ion (i)	Range of C_i (mmol l^{-1})^i	E°" (mV)	S.D. (mV)
Na^+	100 to 140	49.00	0.18
K^+	1.8 to 4.0	77.80	0.20
Ca^{2+}	0.3 to 1.0	140.00	0.14

In measurements of cell A with the SCE as reference, the possible variation of the junction potentials with the composition of the standards must be considered. Taking as example the cell of type A with a sodium indicator electrode and SCE reference, one can write

$$E°' + E_j = E - S \log C_{Na} \qquad (4)$$

where E_j is the algebraic sum of all the junction potentials. The right-hand side of eq. (4) was found to remain constant throughout the calibration range with a standard deviation of only 0.2 mV. This result is important in that it attests to the effectiveness of the constant ionic strength in stabilizing the potentials at the liquid junctions.

5. Emf Measurements at Varying Ionic Strength

Under disease conditions, the ionic strength of serum may vary markedly from its normal value near 0.16 mol l^{-1}. It thus becomes necessary to examine the effect of this variation on the measured electrode potentials. It is then possible to identify and evaluate the errors involved when the electrodes calibrated at one ionic strength are used in the widest range of ionic strengths encountered in the analysis of serum.

The effect of changing ionic strength was studied by preparing three additional sets of solutions. In all sets, the concentrations of K^+ and Ca^{2+} were maintained at their normal levels of 4.0×10^{-3} and 1.0×10^{-3} mol l^{-1}, respectively. The compositions are summarized in table 4. In Set 1, the ionic strength was varied between 0.12 and 0.2 by changing the buffer concentration while the Na^+ and Cl^- concentrations were kept constant at 0.1 mol l^{-1}. In the solutions of Set 2, the sodium ion concentration was constant at its normal value of 0.14 mol l^{-1} and the ionic strength was varied from 0.16 to 0.2 by altering the buffer concentration. In the third set, the buffer concentration was maintained constant and the ionic strength was varied from 0.12 to 0.2 by changing the concentration of sodium chloride.

Table 4. Compositions of solutions of ionic strengths from 0.12 to 0.2[a].

Set No.	C_{Na}	C_{Cl}	C_K	C_{Ca}	I
1	100	100	4.0	1.0	0.12 - 0.2
2	140	100	4.0	1.0	0.16 - 0.2
3	100-180	106-186	4.0	1.0	0.12 - 0.2

[a] C_i in mmol l^{-1}; pH = 7.4 at I = 0.16. All solutions contained NaF at a concentration of 0.1 mmol l^{-1}.

At these ionic strengths, single ion activity coefficients could be calculated with sufficient accuracy for the present purpose by the Debye-Huckel equation in the form

$$-\log y_i = \frac{Az_i^2\sqrt{I}}{1+B\mathring{a}\sqrt{I}} \qquad (5)$$

with the following values applicable at 37 °C: A = 0.5232, B = 0.3316, and $\mathring{a}$ (the ion-size parameter) = 4.6Å.

The emf of cells of the types

Ag;AgCl | Solution | K^+ (ISE) D

Ag;AgCl | Solution | Ca^{2+} (ISE) E

and

LaF_3 | Solution | K^+ (ISE) F

LaF_3 | Solution | Ca^{2+} (ISE) G

was measured for the solutions of varying ionic strength and examined in terms of changes in activity coefficients to be expected from eq. (5). The results indicated that the changes in emf agreed within 0.3 mV with expectations based on the changes in ionic strength and activity coefficients.

In addition, a comparison of data for solutions of Set 1 with those for similar solutions of the same ionic strength in Set 2 showed that replacement of part of the Tris·H^+ by Na^+ resulted in changes in emf no greater than 0.3 mV. Similarly, a comparison of data for Sets 1 and 3 at the same ionic strengths showed that the emf of cells F and G were likewise unchanged at all but the highest ionic strength [11]. Thus there is strong evidence that electrode selectivity parameters have little influence on the potassium and calcium measurements under conditions where a change in ionic strength of serum results from sodium depletion or excess. This conclusion is important in measurements of serum calcium, for many commercial calcium electrodes now in common use are subject to a significantly greater sodium error than is the Simon electrode used in our study.

A further observation is of interest. In the measurements with the SCE reference electrode it was found that the potential at the junction SCE||Solution changes with ionic strength in such a way as to counteract the activity effect. Since most practical measurements are made with calomel reference electrodes, this would tend to reduce the error in the measured concentrations of ions below what would be expected from activity effects alone. Probable errors caused by changes in ionic strength are indicated in table 5. A positive error signifies that the value obtained from the electrode measurement is too high.

Table 5. Probable errors from differences in ionic strength in cells with SCE standardized at ionic strength = 0.16.

I	Na^+ error, %	K^+ error, %	Ca^+ error, %
0.12	+1.2	-1.1	+5.2
0.14	+0.8	-0.4	+3.0
0.16	0	0	0
0.18	+0.8	+0.4	-1.3
0.20	+2.0	+1.1	-2.1

6. Conclusions

While the evaluation of a single ion activities in biological fluids remains a problem of considerable complexity, measurements in synthetic electrolyte mixtures simulating serum indicate that ion-selective electrodes respond in a near-Nernstian manner to changes in the *concentrations* of the species for which they are selective. At a constant ionic strength of 0.16 mol l^{-1}, the activity coefficients and liquid-junction potentials are reasonably constant. Effects of changes in ionic strength on the measured cell emf are relatively minor and almost entirely accounted for by alterations in the activity coefficients. These results lead one to expect that standards similiar to the ones described here may be well suited for the calibration, on a concentration basis, of ion-selective electrodes for use in biological or clinical media. The lanthanum fluoride electrode appears to be promising as a reference electrode, provided account is taken of the effect of ionic strength on the activity coefficient of the fluoride ion.

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ELECTROLYTE ACTIVITIES IN HUMAN BLOOD PLASMA

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1. Introduction

Assessment of a patient's acid-base and electrolyte status is an essential diagnostic procedure in many acute and non-acute diseased states. Diseases of the renal and respiratory systems primarily result in acid-base and electrolyte imbalance although other regulatory mechanisms exist [1][2]. Care of the critically ill patient may require frequent measurement of blood gases and electrolytes during potentially life-threatening episodes, *e.g.*, in cardiac surgery. The use of serum or plasma electrolyte measurements to evaluate the condition of acutely ill patients reflects the fundamental importance of extracellular electrolyte concentrations in basic physiological processes.

From a physico-chemical standpoint blood plasma is an extremely complex liquid. It is moderately concentrated in inorganic salts and contains appreciable levels of polyelectrolytes (proteins). Uncharged species, *e.g.*, CO_2, glucose and urea, are present and have important effects on metabolic processes. Because of the complexity of this chemical system, the physician bases his clinical judgments on measurements of total electrolyte concentration. Indeed, he has had little choice as the analytical methods in routine use in clinical laboratories provide only concentration measurements for Na^+, K^+, Cl^-, and HCO_3^-. The total concentrations of these species give little information on the true composition of plasma, which is influenced by specific ion-ion interactions (metal-ligand complex formation) and non-specific interactions characteristic of non-ideal solutions. The physical chemist prefers to define for each species present an "activity" which may crudely be regarded as a concentration term, corrected for the various specific and non-specific interactions.

The complexity of human plasma electrolyte composition has been recognized most clearly in the measurement of ionized calcium [2]. The preferred practical method of making this measurement uses an ion-selective membrane electrode [3]. Such electrodes are unique among analytical sensors in that they have a response determined by ionic activity, rather than total concentration. Routine serum electrolyte measurements in many clinical laboratories are now made with ion-selective electrodes used in the Technicon Instrument Corporation systems SMAC and Stat-Ion. These systems dilute the serum sample so that ionic interactions are controlled, and the results are obtained as concentrations. There have been several reported applications of ion-selective electrodes in the direct measurement of electrolytes other than ionized calcium in whole blood, plasma and serum. These have included the determination of sodium and potassium [4-7] as well as descriptions of more complete systems for acid-base and electrolyte measurements [8-11].

The use of ion-selective electrodes in whole blood, serum or plasma as opposed to dilute serum presents numerous problems, not least of which is an understanding of what is actually being measured. Rational interpretation of the results of electrode measurements requires a knowledge of the true nature of each ion species measured. Previously, Walser [12] and Robertson [13] have considered ion interactions in plasma ultrafiltrates.

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[2]Figures in brackets indicate literature references at the end of this paper.

Dahms *et al.* [14] have discussed the activities of sodium, potassium and chloride in human serum as determined by electrode. In this paper a model is developed for electrolyte ion activities in human plasma. A complete model would include acid-base, metal-ligand complex, and redox equilibria as well as those involving gas and solid phases. However in order to contain the complexity of the problem, only a limited range of acid-base and complex equilibria will be considered.

2. Development of the Model

This model is intended to represent the extracellular fluid in equilibrium with erythrocytes and other blood cellular components, *i.e.*, the plasma fraction of blood. The term plasma implies *in vivo* plasma rather than the *in vitro* counterpart which contains an anticoagulant. However, because many of the equilibria constants required have only been measured at 25 °C, numerical results are obtained at this temperature; this does not detract from the accuracy of the model but only from its interpretation.

A. Water content of plasma

The solids, particularly proteins dissolved in plasma occupy a significant fraction of the total plasma volume, *i.e.*, the partial molar volume of proteins is not negligible. An alternative statement is that the activity of plasma water is less than one. Activities may be given in two different scales; molar (mol/l of solution) and molal (mol/kg of solution). The molar scale is adopted here mainly because it is in agreement with normal clinical chemistry practice (meq/l, mg/dl, *etc.*).

McLean and Hastings [15] have given the water content of plasma as a function of the protein concentration (in g/dl):

$$H_2O/100 \text{ ml plasma} = (100-1) - 0.75\ P \tag{1}$$

Accordingly, the total concentration of all non-protein species is corrected from mol/l of plasma to mol/l of plasma water using the factor given by eq (1).

B. Specific ionic interactions

This model is restricted to consideration of the clinically significant cations H^+, Na^+, K^+, Ca^{2+}, and Mg^{2+} and their interactions with various anions; metal-protein interactions will be considered below. It is necessary to restrict the range of anionic species considered; table 1 shows the anions which are thought to represent the majority of those present in plasma. A great many others, *e.g.*, amino acids, ascorbic acid, *etc.*, could have been included but were omitted to simplify the model. As a further simplification, not more than one acid-base dissociation was considered for each acid as shown in table 1; this is a reasonable restriction, from comparison of the acid pK with physiological pH values. In general terms, the model considers the formation of a 1:1 complex between each metal and each anion. No polynuclear complexes are considered, *e.g.*, of the type M_2L, nor are mixed complexes, *e.g.*, M_1M_2L. Not all of the possible 1:1 complexes were included as for some the stability constant is too small to measure, *i.e.*, the complex is completely dissociated; table 2 lists the complexes included.

For each ligand:

$$L_1 \rightleftharpoons L_2 + H^+ \qquad K_a = \frac{[H][L_2]}{[L_1]} \tag{2}$$

$$M_i + L_1 \rightleftharpoons M_iL_1 \qquad K_{1i} = \frac{[M_iL_1]}{[M_i][L_1]} \tag{3}$$

$$M_i + L_2 \rightleftharpoons M_iL_2 \qquad K_{2i} = \frac{[M_iL_2]}{[M_i][L_2]} \tag{4}$$

Table 1. Plasma anions and ionization constants.

		pK[a]
Cl^-		- [b]
$CO_2 + H_2O$	----- $HCO_3^- + H^+$	6.35
$H_2PO_4^-$	----- $HPO_4^{=} + H^+$	7.21
HSO_4^-	----- $SO_4^{=} + H^+$	1.99
$HCitrate^{=}$	----- $Citrate^{\equiv} + H^+$	6.49
Lactic acid	----- $Lactate^- + H^+$	3.86
Pyruvic acid	----- $Pyruvate^- + H^+$	2.49

[a]Numerical values from [16].
[b]Chloride is assumed to be completely ionized.

Table 2. Complexes and dissociation constants pK.[a]

	HCO_3^-	$H_2PO_4^-$	HPO_4^{2-}	SO_4^{2-}	$HCit^{2-}$	Cit^{3-}	$Lactate^-$	$Pyruvate^-$
Na^+			-1.11	-0.72		-0.93		
K^+			-1.00	-0.96		-1.10		
Ca^{2+}	-1.26	-1.08	-2.70	-2.31	-3.09	-4.85	-1.42	-1.08
Mg^{2+}	-1.16	-1[b]	-2.50	-2.25	-2.46	-4.57	-1.37	-1(2)

[a]Numerical values from [13] and [16].
[b]Estimated values.

where [ ] denotes activity. The total concentration of the ligand is:

$$L_{tot} = [L_1]/\gamma_1 + [L_2]/\gamma_2 + \sum_i [M_iL_1]/\gamma_{M_iL_1} + \sum_i [M_iL_2]/\gamma_{M_iL_2} \quad (5)$$

where γ represents an activity coefficient. From (2) - (5):

$$[L_2] = \frac{L_{tot}}{[H]/K_a\gamma_1 + 1/\gamma_2 + \sum_i K_{1i}[M_i][H]/K_a\gamma_{M_iL_1} + \sum_i K_{2i}[M_i]/\gamma_{M_iL_2}} \quad (6)$$

$$[L_1] = [H][L_2]/K_a \tag{7}$$

$$[M_iL_1] = K_{1i}[M_i][L_1] \tag{8}$$

$$[M_iL_2] = K_{2i}[M_i][L_2] \tag{9}$$

Equations (6) to (9) can be solved given L_{tot}, H, M_i and the activity coefficients. The values of L_{tot} and H (*i.e.* pH) are assumed known, as are the total concentrations of each metal ion. The mass balance condition for each metal ion is given by:

$$[M_i] = \left\{M_{tot} - \sum_j [ML_j]/\gamma_{ML_j}\right\} \gamma_{M_i} \tag{10}$$

Solution of equations (6) to (9) is obtained iteratively using an initial estimate for M_i and calculated activity coefficients to give an improved estimate for M_i from (10). The method of calculating activity coefficients and details of the computer program used to obtain the solution are given below.

C. Protein-ion interactions.

This model is restricted to a consideration of the interaction of calcium and magnesium with albumin and globulin. The effect of chloride binding to albumin, although included in an earlier treatment [14] is not considered here as the magnitude of such interactions has been shown to be negligible at physiological pH [17,18].

There have been many studies of calcium binding to albumin, the results of which have not always been in agreement. Recently, Pedersen [19] has shown that under physiological conditions, the concentration of bound calcium is given by

$$\frac{(CaAlb)}{Alb_{tot}} = \frac{12\ K_1(Ca^{2+})}{\left\{1 + K_1(Ca^{2+}) + 10^{-pH}\ K_a\right\}} \tag{11}$$

where $K_a = 10^{7.55}$, K_1 = 241 l/mol, and ( ) represents concentration. This equation is used in the model. Pedersen [19] also studied the binding of calcium to γ-globulin. Although far less data is available for globulin binding, it was shown that the binding was less and of smaller pH dependence. A linear regression to the data of table 2 [19] gives the equation.

$$(CaGlob)/Glob_{tot} = 0.564\ pH - 1.79 \tag{12}$$

at a mean ionized calcium concentration of 3.8 mmol/l and a globulin concentration of 0.13 mmol/l. Pedersen has given insufficient data to estimate a reliable equilibrium constant for this reaction and, therefore, the approximation is made here that under physiological conditions the concentration of globulin bound calcium is linearly proportional to the free calcium ion concentration.

Relatively few studies have been made of magnesium binding to albumin. A recent treatment by Frye *et al.* [20] shows that the equilibrium follows a pH dependent mass law

relationship. The data of table 2 [20] can be reduced by linear regression to show the pH dependence of the Mg-albumin complex stability constant:

$$K_1 = 142.65\ pH - 952.79. \tag{13}$$

Assuming six ion binding sites per molecule of albumin,

$$(MgAlb) = \frac{6\ K_1(Mg^{2+})Alb_{tot}}{1 + K_1(Mg^{2+})}\ . \tag{14}$$

No data has been found on magnesium binding to globulins and, therefore, the assumption is made here that calcium and magnesium bind identically to γ-globulin.

The treatment used in this model assumes that calcium and magnesium do not compete for the same binding sites on albumin or globulin. Data on the competitive binding of these two metal ions to proteins does not appear to be available. However, it must be noted that under normal physiological conditions not all binding sites are occupied and therefore the assumption of independent binding of calcium and magnesium may not be unreasonable.

D. Activity coefficients

Activity coefficients for all uncharged species were assumed to be 1, as were the activity coefficients of proteins and metal-protein complexes. The activity coefficients for all other ionic species were calculated by means of the Davies equation [21]:

$$-\log\gamma_i = 0.509\ n_i^2\ \frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I \tag{15}$$

where n_i is the ion charge and I the ionic strength. This equation takes no account of the individuality of ionic activities nor is it particularly accurate for trivalent species. However, only one such ion (citrate) was included, and at low concentrations, so the equation was considered adequate.

Following the convention of Scatchard [18], the ionic strength was considered to be a function only of non-protein ions but was otherwise defined conventionally;

$$I = 0.5\ \Sigma\ n_i^2 \cdot C_i \tag{16}$$

where C_i is the ion concentration.

3. Computer Simulation of the Model

Figure 1 shows a flow diagram of the computer program used for numerical simulation of the model. The program is written in Tymshare Fortran IV; full details may be obtained from the authors.

Input data to the program includes: pH; Na, K, Ca, Mg, total concentrations; albumin and globulin total concentrations; the total concentrations of each of the seven anions given in table 1. Initial values for the ionic strength (equal to the sodium ion concentration) and free metal ion activities are set. The estimates for the monovalent cations are obtained from the products of the concentrations and the activity coefficients derived from the estimated ionic strength. Estimates for divalent ion activities were obtained by trial and error; for normal plasma these values were set equal to half the product of concentration and the estimated divalent activity constant.

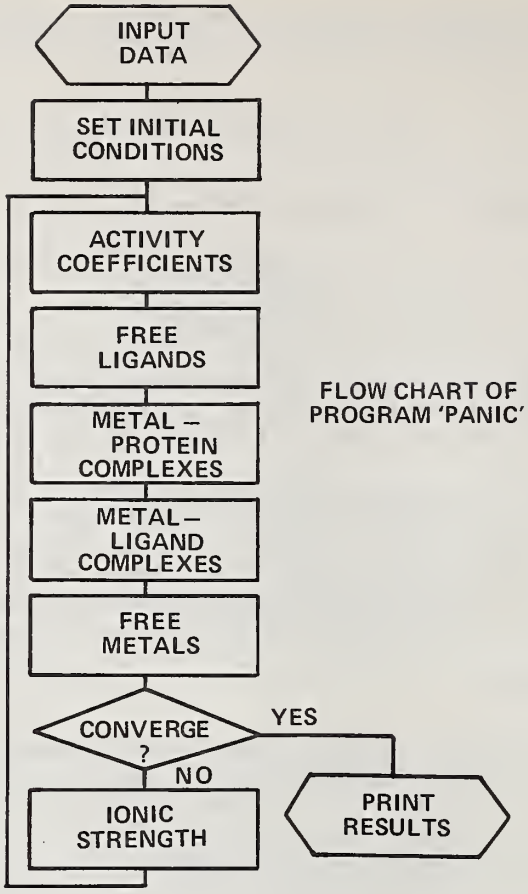

Figure 1. Flow diagram of computer program used to simulate model.

The program then proceeds to solve equations (6) to (9) to calculate the free ligand and metal-ligand complex activities. Next, the concentration of protein bound metals are calculated using equations (11) to (14). At this point it is possible to make a better estimate for the free metal ion activities using equation (10). The program now tests for convergence by comparing the initial estimates. If these differ by more than 1 percent, the free metal ion activities are reset to the mean of the initial and new estimates, and the calculations repeated. In this iterative fashion a solution is usually quickly obtained to the set of equations.

4. Results and Discussion

A real advantage of a model of a system as complex as blood plasma is that it allows the effects of variables to be investigated when these may be experimentally inaccessible. Thus, it is possible to examine the properties of plasma when all the components take extreme high or low values; clinically such a situation is extremely unlikely. Table 3 shows the calculated ionic strengths and activity coefficients in such hypothetical plasma samples compared to normal values. Monovalent activity coefficients are in the range 0.76 ± 0.02 and divalent coefficients in the range 0.33 ± 0.03. The constancy of the activity coefficients over the extreme physiological range indicates that either an activity or a concentration scale may be used to obtain medically significant results. In the particular case of ion-selective electrode measurement of electrolytes, it is possible to calibrate the electrodes on a thermodynamic activity scale or on a concentration scale by appropriate choice of standards at a physiological ionic strength [22]. It must be noted that significant differences exist between concentrations expressed in terms of plasma

Table 3. Comparison of normal and abnormal plasma.

Input data (total concentration)	Low	Normal	High
pH	7.0	7.4	8.0
Na meq/l	120	140	170
K meq/l	2	4	8
Ca mg/dl	5	10	15
Mg mg/dl	1	2.5	5
Albumin g/dl	2	4.7	8
Globulin g/dl	1	2.5	5
Cl meq/l	70	100	130
CO_2 meq/l	10	30	40
P mg/dl	1	3.7	10
S mg/dl	0.5	0.9	1.5
Citrate mg/dl	2.4	2.4	2.4
Lactate mg/dl	10	10	10
Pyruvate mg/dl	0.6	0.6	0.6
Calculated values			
Ionic strength	0.108	0.152	0.204
Monovalent activity coefficient	0.778	0.759	0.746
Divalent activity coefficient	0.365	0.332	0.310

volume and plasma water volume. Table 4 shows the effect for the four commonly determined electrolytes. Calibration of an electrode with aqueous protein-free standards results in plasma measurements on the plasma water volume scale.

The model predicts the activities of 46 components of plasma; table 5 shows the calculated distribution of these in normal plasma. It is also possible to investigate the effect of one variable on others, *e.g.*, the pH dependence of ionized calcium and magnesium (fig. 2). These results indicate possible applications of the model; however, it is not intended to explore these in detail here.

Table 4. Comparison of normal plasma electrolyte values on different scales.

	Total concentration meq/l plasma	Ion concentration meq/l plasma H_2O	Ion activity meq/l plasma H_2O
Na	140	149	113
K	4	4.3	3.2
Cl	100	107	81
CO_2	30	30[a]	23[a]

[a] HCO_3 only.

Table 5. Concentrations of normal plasma components in mol/l plamsa H_2O.

H^+	5.24×10^{-8}	Na^+	0.149	K^+	4.26×10^{-3}	Ca^{2+}	1.26×10^{-3}	Mg^{2+}	6.46×10^{-4}
Albumin	6.82×10^{-4}					CaAlb	9.14×10^{-4}	MgAlb	2.54×10^{-4}
Globulin	1.56×10^{-4}					CaGlob	1.24×10^{-4}	MgGlob	6.31×10^{-4}
Cl^-	0.107								
CO_2	2.01×10^{-3}								
HCO_3^-	2.97×10^{-2}					$CaHCO_3^+$	2.26×10^{-4}	$MgHCO_3^+$	9.29×10^{-5}
$H_2PO_4^-$	1.79×10^{-4}					$CaH_2PO_4^+$	9.01×10^{-7}	$MgH_2PO_4^+$	3.83×10^{-7}
$HPO_4^=$	6.33×10^{-4}	$NaHPO_4^-$	4.04×10^{-4}	$KHPO_4^-$	8.97×10^{-6}	$CaHPO_4$	4.42×10^{-5}	$MgHPO_4$	1.42×10^{-5}
HSO_4^-	3.90×10^{-10}								
$SO_4^=$	2.29×10^{-4}	$NaSO_4^-$	5.96×10^{-5}	KSO_4^-	2.96×10^{-6}	$CaSO_4$	6.51×10^{-6}	$MgSO_4$	2.90×10^{-6}
$HCit^=$	7.61×10^{-7}					CaHCit	1.31×10^{-7}	MgHCit	2.06×10^{-8}
$Cit^{\equiv}$	2.45×10^{-5}	$NaCit^=$	5.99×10^{-5}	$KCit^=$	2.52×10^{-7}	$CaCit^-$	8.05×10^{-5}	$MgCit^-$	2.16×10^{-5}
HLac	2.56×10^{-7}								
Lac^-	1.17×10^{-3}					$CaLac^+$	1.29×10^{-5}	$MgLac^+$	5.86×10^{-6}
HPyr	6.78×10^{-10}								
Pyr^-	7.26×10^{-5}					$CaPyr^+$	3.66×10^{-7}	$MgPyr^+$	1.55×10^{-7}

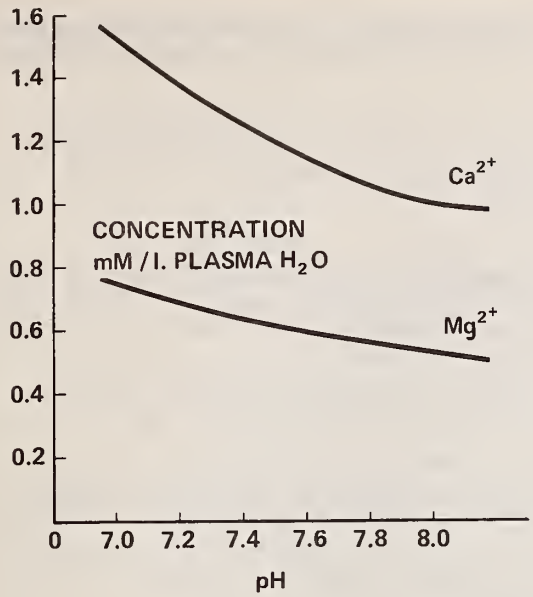

Figure 2. Calculated pH dependence of ionized calcium and magnesium in normal plasma.

It may be argued that the model is incomplete in that it does not require electroneutrality--a condition which is always satisfied for an actual plasma sample. The absence of this condition is intentional and, in fact, it is not required of the model. Electroneutrality is obtained at a given pH by charge balance between the proteins and other ions. Perhaps the most serious criticism of the model is that it relies entirely on published equilibrium constants, the accuracy of which is not always beyond question. If it serves no other purpose the model emphasizes the need for reliable thermodynamic measurements of these constants at various temperatures and at physiological ionic strength. It is to be hoped that this treatment of ionic equilibria will lead to a clearer understanding of the species present in blood plasma and their role in metabolic processes.

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THE KING'S COLLEGE HOSPITAL ION-SELECTIVE ELECTRODE SERUM ELECTROLYTE ANALYZER

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In recent years there has been a great increase in the availability of ion-selective electrodes, and these are finding wider use in routine clinical chemistry laboratories (table 1). The obvious difficulty is that such electrodes measure activity which must be somehow related to concentration by calibration and conversion factors. Early reports, *e.g.*, with the Na electrode suggested that the conversion factor might vary in disease states when abnormal concentrations of protein were present [1][1].

Table 1. Some ion-selective electrodes.

Cations	Anions
H^+ (pH)	F^- Cl^-
NH_3 (NH_4^+)	Br^- I^-
Na^+ K^+	CN^- NO_3^-
Ca^{2+} Cd^{2+}	
Cu^{2+} Pb^{2+}	

We felt that it would be an interesting and potentially useful experiment to construct a multi-channel continuous-flow serum electrolyte analyzer based entirely on ion-selective electrodes. This would dispense with the need for chemical reactions to first make a colored solution, pass light through it, measure what emerges and then by complex electronics, convert it to the concentration of the substance being measured.

Table 2 shows the general principles adopted in the analyser. The Na electrode is probably the most critical part of the system. The Na electrode is based upon an alkaline

Table 2. General outline of KCH analyzer.

Na^+ electrode:		acid-serum dialyzed into high-osmolar alkaline buffer
K^+ electrode :		Na^+ activity backed-off
HCO_3^-	:	pH electrode CO_2 from acidified serum diffuses into Na_2CO_3
Glucose	:	O_2 electrode. High concentration glucose oxidase causes quick fall in PO_2
Urea	:	NH_3 electrode. Urease → NH_3 from urea

[1]Figures in brackets indicate the literature references at the end of this paper.

error in a glass pH electrode. If serum is made alkaline with buffer, then protein interference can be a problem. By dialyzing an acidified serum sample into an alkaline solution of high molarity, a linear response to changes in Na concentration can be obtained. Figure 1 shows the flow-diagram for the analyzer.

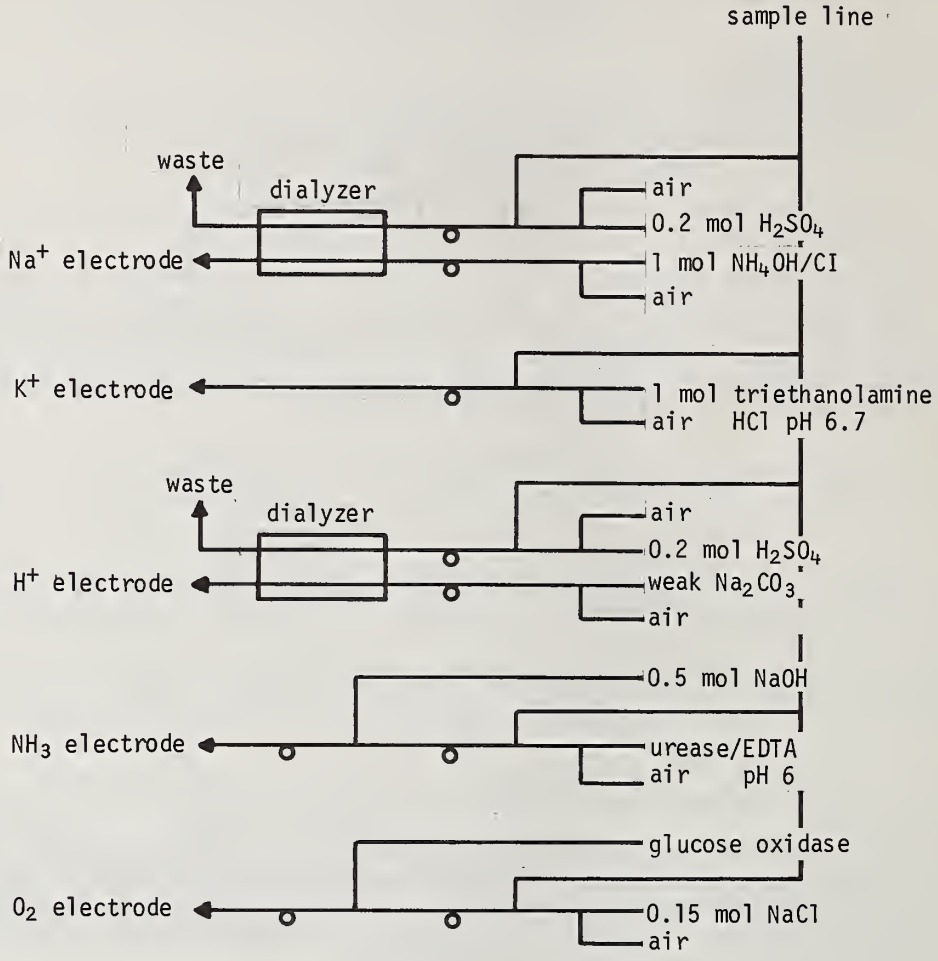

Figure 1. Flow-diagram for KCH Ion-selective electrode serum electrolyte analyzer.

The K-selective glass electrode is responsive to an appreciable extent (activity ratio about 10:1) to Na ions, but fortunately the Na response is linear and additive. Accordingly, K is measured after Na by means of time-delay coils and the Na contribution from the Na electrode is electronically backed-off from the total ion activity. The serum HCO_3^- measurement is very simple. About 50 percent of the CO_2 liberated from acidified serum can be dialyzed across a standard membrane into a Na carbonate buffer whose pH alters with varying CO_2 content.

The measurement of glucose by an O_2 electrode gave trouble at first owing to the O_2 content in the air-segmentation bubbles in the mixing coils. It was not found necessary, however, to use inert gases for this purpose. By using a high concentration of glucose oxidase, the rapid fall in PO_2 found in the first 30 seconds was found to be proportional to the glucose concentration. The urea method is potentially as accurate a method as it is possible to devise and should be superior to the non-specific diacetyl monoxime reaction.

The urease reaction takes place at a slightly acid pH after which the sample is made highly alkaline (pH 12) before being fed through the NH_3 electrode.

The electrolyte analyzer has been assembled at King's College Hospital from commercially bought electrodes, sampler, pump, dialyzers, mixing coils, heated water bath, recorder and electronic components. Unfortunately at the time of writing, it is not yet in routine operation although all the individual channels have been worked up prior to the final design of the machine. Figures 2 and 3 show general views of the analyzer.

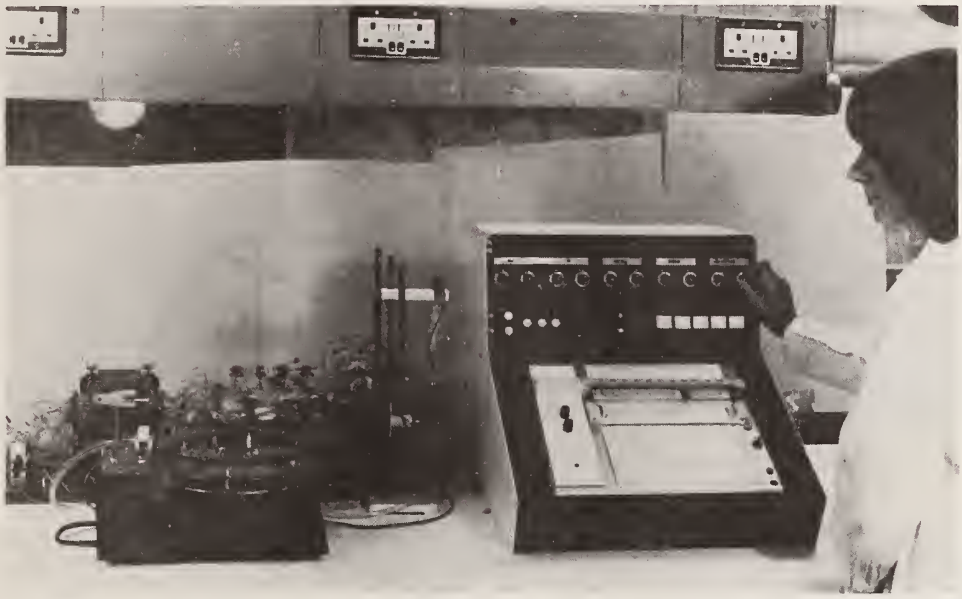

Figure 2. View of KCH analyzer.

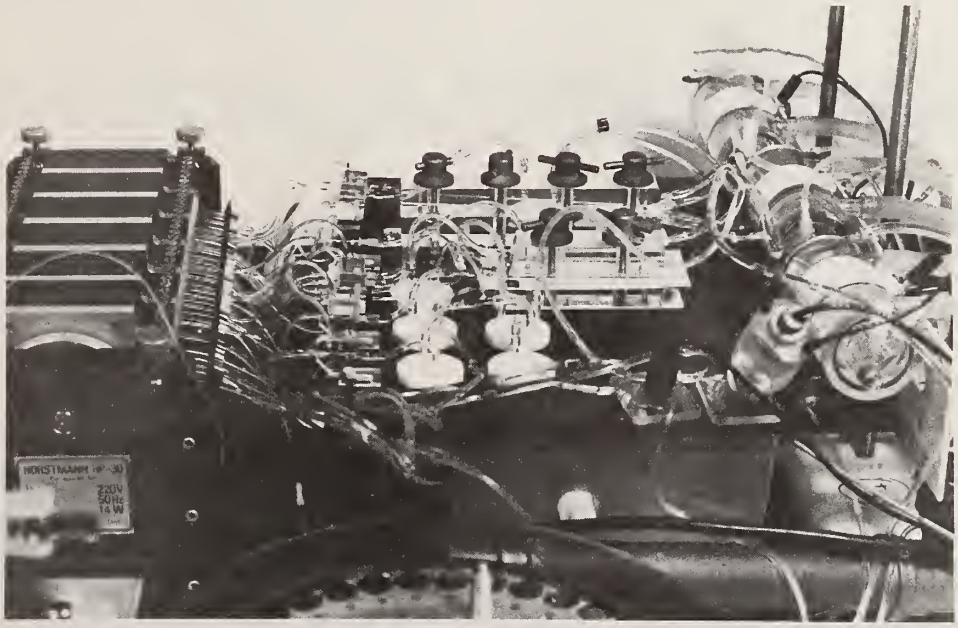

Figure 3. Close-up view of analyzer.

All the arguments that have raged for 60 years about the measurement of H^+ concentration or activity apply in some measure to ion-selective electrodes. However, since no one in practical terms really wants to measure or report urea as "pNH_3" or K^+ as "pK" [2], then we will all make the necessary effort to adapt in a reasonable manner the activity measurements to concentration units. All our routine methods have inherent errors or compromises of one sort or another. We really do not see any good reason at all why we cannot also make an effort with regard to the pH-glass electrode and use a single unified set of units such as the SI provides for all our common electrolyte and blood gas analyses.

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The transcription of selected segments of the discussion sessions is included in this volume because it supplements much of the material in the preceding chapters. In this chapter will be found additional comments on practical problems, instrumentation, methodologies, and concepts on the subject of blood pH, gases and electrolytes.

The workshop was arranged to provide time for discussion at the conclusion of each series of lectures on a particular subject. The following discussions were transcribed with a minimum of editing and represent the spontaneous exchange of remarks of the various participants as they were recorded at the workshop. The speakers have had an opportunity to correct the transcription of their remarks for technical accuracy.

Bates:

Before this session began, several of us were reminiscing about the meeting in 1964 at the New York Academy of Sciences and I recall in 1964 there was a great deal of discussion about two things, one of them being the validity of so-called "unlogging" the pH value, and the other one was concentration versus activity, in other words, concentration versus excess free energy. It seems rather remarkable that in eleven years the utility of one of these hasn't proved itself. We still have the same problem of which to use, and it occurs to me that this might be due to the fact that the experimental methods for determining pH, for example, do depend more closely on an activity than on concentration, and I wonder if this is the reason that this dilemma still faces us.

Cohen:

My view of this is that this is not a dilemma but a sematics difference. When hydrogen ion concentration is spoken of, at least in the parlance of clinicians and physiologists, I think what is meant is "the negative antilogarithm of the pH" which is, as you point out, an activity measurement. This is not to say that one is ignoring or brushing aside the difference between concentration and activity, but that one is simply using a shorthand notation to designate measured quantity in terms that are more readily conceptualized. And I am wondering whether you would view this as an acceptable "derivation" from the measurement of pH or whether we ought employ a different term for it.

Bates:

Well, it seems to me that we are really begging the question of whether it's an activity or a concentration that is crucial in biochemical and biological processes, aren't we?

Cohen:

Well, that is what I mean to imply. That is, I don't think anybody really quarrels with the notion that it is the activity that is fundamentally at issue, that biological systems and chemical systems must be responding to the chemical activity. That being the case then, I think it's a matter of arbitrary definition as to what one choses to call the unit of measure as long as it serves the need for unambiguous communication.

Austin:

I think that perhaps it may be the fact that pH is essentially a "dimensionless" factor that is important. pH serves as an indicator in a given situation, and it avoids the issue of applying other than a unit dimension. Is it a nano-equivalent? How does that fit with a milli-equivalent? I think that is one important factor, and it speaks for the continued use of pH. We are dealing with rather gigantic differences when we're talking about milli-equivalents versus nanomoles. One can not argue that we should then speak of the log of the sodium or potassium concentration, since we administer these in their actual concentrations, even though their biological activity is what is important. Though we give bicarbonate in mEq to correct hydrogen ion concentrations/activities, no one corrects acidosis, for example, by hanging a bottle of H^+.

Weisberg:

I have two thoughts on pH versus the hydrogen ion concentration. Physicians have degraded themselves by saying they can't understand pH, whereas chemists as a group use pH. We're not going to be able to change the rest of the chemical field by saying we're going to use hydrogen ion concentration. Secondly, I find that those who speak for the hydrogen ion concentration are really deluding themselves because a change of 10 nanomoles in a hydrogen ion concentration has a completely different interpretation when it's on the highly alkalotic side versus the highly acidotic side. So you're going to give a greater importance to one nanomole of hydrogen ion where the effect on the body enzyme activity, and so on, is completely different. So my own feeling is that I give both pH and hydrogen ion concentration in my report on the diagram; you can use whichever you will. But if you realize, as most of us do, that a pH unit change is a tenth or ten times going the other way, we can get that concentration effect the same way. We're dealing with numbers, but we're giving undue importance to a number of the hydrogen ion concentration.

Cohen:

Well, I think one could find as many examples of biologic relationships that would be linear with respect to hydrogen ion concentration as you could with respect to pH. I don't really think the argument that enzyme systems or other things are responding linearly to pH rather than to hydrogen ion concentration or activity is a very compelling one. I think the only compelling argument, at least in my mind, for retaining, or perhaps substituting, the notation, "hydrogen ion concentration", (with the caveat that I mentioned earlier) is that it simplifies the mathematics that describe the bicarbonate-carbonic acid buffer system. That is the only justification that I can see, but it seems to me that is one that can't be minimized. It has some very useful pedagogical and practical advantages.

Engel:

I understand Dr. Bates' surprise because it has taken a long time since we started these discussions at the meetings at New York Academy of Sciences. I think also that you should realize that the people working in this field of acid-base physiology are a very inhomogeneous group of people. We have pure chemists, we have physiologists, we have medical doctors in the ward, etc, and the interests of the different groups of people are very different and, therefore, the emphasis is sometimes put on one side of the aspect. The other thing I would like to comment on is the old questions around the "great trans-Atlantic acid-base debate" which was brought up again. I don't think it has ever stopped even though it sort of dampened for a few years. I think, however, that we should agree to divide our problems in three different groups. First, we have some basic definitions which apply to clinical chemistry as well as to pure and applied chemistry. I think it's very feasible to use these very well systematized and well defined concepts. Second, as clinical physiologists and as acid-base physiologists, we might be interested in some derivatives of these well-defined and measurable variables and, of course, we would like to define some variables or derived variables which make physiological sense. And thirdly, we have the problems related to the clinicians with pathophysiological interpretation of these either derived or basically measured values. I think that we have been very good in the past of mixing all three of these groups together in one melting pot, and that lots of the questions which were discussed during the "great trans-Atlantic acid-base debate" came about because of this maxture of what our different goals and definitions were.

Howorth:

I'd like to go back to something that people were talking earlier on about pH as opposed to so-called concentration units. I think many physicians in practical terms need some sort of concentration unit. I mean if a surgeon is aspirating gastric juice, he likes to have some measure of how much acid is being removed from that patient. You also need a quantitative measure with renal excretion of H^+ and renal function tests. In everyday practical medicine we need to know how much acid a patient is putting out in his urine on a quantitative basis. This is where the *mole* as the amount of substance is superior to the dimensionless pH concept.

Cohen:

Is it not true that in other systems where the measurement is done electrochemically, for example, calcium, we report the substance in concentration units when we recognize that we are measuring it electrochemically? I don't think that poses a problem.

Noonan:

Well, I think a fundamental concept in analytical chemistry is the idea of recoverability. I can say that I am measuring glucose quantitatively if I can recover a certain amount of pure glucose which has been added to it. The glucose technique is directly measuring the concentration. However, I cannot take a certain quantity of hydrogen ion and add it to a solution and measure it with an electrode and say that it is concentration because concentration is not being measured. In terms of ion-selective electrodes for sodium and potassium, and indeed pH, you can get some constant correlation between the activity and the concentration that will work in many, many cases but will not work in all cases. If you really want to be precise, there should be a difference between a sodium measurement that's made with an electrode and a sodium measurement that's made with a flame photometer.

Austin:

I agree, sodium concentration is important when you are replacing it, but with pH, when considering dissociation equilibria (Davis, R. P., *Amer. J. Med.* 42, 159-162, 1967), the quantity of hydrogen ion that you're measuring is so much smaller that the quantity of sodium. They are of an entirely different magnitude. This may create some difficulties with measuring diminutive quantities of hydrogen ion where we're measuring an entirely different number of sodium and potassium ions. How does this help you? Apart from concentrations, pH denotes a more meaningful description of the acid-base equilibrium reaction. If we are talking about acid-base balance that's one thing; ionic concentrations or activity are another matter. Recall in the Henderson-Hasselbalch equation that we do take the log of the bicarbonate concentration, even though we give bicarbonate in its sodium form for practical purposes.

Gambino:

I think I'd like to say that I agree basically with Siggaard that it is preferable to go to some type of measurement or some type of indication of the amount of energy and the quantity of substance, but I'd like to have some measure of the energy. I think it's important to point out that we cannot utilize the suggestion that the way we make the measurement should determine the unit. For example, in temperature we estimate the average kinetic energy, we measure the movement of a mercury column and we measure current in a system. But we don't attempt to say that because we measure current that we should then define temperature as an estimate of kinetic energy in terms of current. We don't do that. We go back to some fundamental quantity such as the joule. I think we should try to get a quantity into these fundamental units.

Durst:

There's something that I picked up from our discussions that I might just mention now. That is that we're using the word "derived" in two different ways. One is in reference to the SI system where derived means units expressed as products or ratios of the base units without numerical factors as opposed to what we are calling derived units such as bicarbonate, that is to say, a parameter calculated from a measured parameter or parameters. Again, this in one more example of a semantic problem which could be significant.

Weisberg:

May I add one more semantic problem. You're using the word nomogram erroneously. I have collected 105 diagrams, 17 nomograms, and 12 slide rules. The first diagram goes back to 1914, and I think we are really referring to diagrams rather than nomograms. Dr. Malenfant had a true nomogram. A Cartesian nomogram and a diagram are two completely different things. A diagram does have benefits for a practitioner to see, especially with continuing sets of data. One set of data means nothing; a point in time of compensation or uncompensation. But

if you can tell where the patient has been or where the patient is going and thus follow that on a flow chart. Dr. Siggaard-Andersen had, of course, the time chart going along with that. So I think we ought to be clear that we are speaking of diagrams most of the time for physicians to look at. The nomogram is a laboratory device to save ourselves some mathematics.

Howorth:

I think a nomogram is something that one uses to derive a figure from primarily observed data. A parameter isn't something which is directly measured, it's something that's indirectly measured.

Visser:

Well, there will be no confusion in this case between "derived" and "derived", because base excess is a derived quantity, but maybe another derived quantity can be expressed in a basic unit. So we must distinguish between the quantity and the unit.

Cohen:

I'd just like to comment on Dr. Engel's presentation. I had two commentaries to make; one trivial, really, and the other perhaps more substantive. The trivial one being that the use of the term "net acid" in his scheme, if I understood it, is somewhat at variance with modern definitions of acids and bases; it doesn't seem to conform to a proton donor. The second is the issue of simplicity. I think there is nothing internally inconsistent about that system for describing, in phenomonological or empiric terms, what happens to living organisms when they are challenged. The only quarrel I have with it is that it seems to me to be unnecessarily complicated. One of the issues that surfaces in my mind, as a dominant theme, relates to the fact the system with which we are dealing is inherently complicated, simply by virtue of its physiology being so multifactorial. We are, therefore, better served by the conceptual framework for discussing that system's behavior which is as simple as we can make it. I don't see any compelling justification or reason for a system, such as described, in contradistinction to one which deals just with the carbonic acid/bicarbonate equilibrium and describes what happens to the organism in those very simple terms.

Engel:

If I can take the last comment first, then if you did total body studies of acid-base metabolism, you would very quickly run into the same problems which we have been running into. That is, you can't describe them with the set of variables we have been using so far. When you define the body as a system, I think you should make it just as complicated as is needed in order to completely describe the system. This is exactly why we wanted divide titratable acid up in three components: Carbonic acid which is regulated by the lung-brain system, metabolizable acid which is regulated by the metabolism, and the net acid for which the only route of escape is the kidney, and this makes physiological sense. I don't know whether the scheme for the breakdown was too complicated or not, but anyway we feel that this is the only way where you can go in and put a finger on the physiological mechanisms and causes which are involved in an acid-base disturbance. One thing more is that you may measure, as I said, a normal acid-base status in the blood and yet have a very pronounced acid-base disturbance in terms of balance studies.

Cohen:

I don't think there is any question about this latter point. Clearly, we can have a very markedly disturbed external balance without having the deviation.

Engel:

Right, but you won't find out unless you carry out the balance measurements.

Cohen:

Of course, but let me just take those two issues in turn. One is that I think it is possible to designate external balance of acids and bases without utilizing that breakdown

factor of acidity in the classic context of the net acid balance. In other words, there is a certain rate of net acid excretion, by the kidney, which we all recognize as ammonia plus TA minus the bicarbonate that is excreted. In the steady state, this balances the rate of endogenous acid production. Now, there is no satisfactory way, nor do I think your system provides me, for quantitating directly the rate of endogenous acid production. Given a diet, one can't use that scheme, for example, to specify what its net contribution of hydrogen ion or base will be to the body fluids, any more than one can take a diet and analyze it for this purpose in any other way. So one is left with a conundrum that's been an issue in physiology from the beginning of time; that is, unless one constructs a purely artificial diet which can be demonstrated to be complete metabolized, à la Relman, external hydrogen ion balance must be estimated indirectly since we have no handle on the rate of endogenous acid production. For this purpose, we use the rate of net acid excretion by the kidney, measured in terms of hydrogen ions which are gaining access to the urine, as a fix on that parameter.

Engel:

Well, I think that you can assess the endogenous net acid production. But there is a discrepancy between what we and you call net acid. Net acid in our terms is defined as the titratable value (TA) to an endpoint at pH 7.40 and temp. = 37 °C when carbonic acid (CA) and metabolizable acid (MA) concentrations are zero. This is the operational definition and it gives you values which are quite different from the net acid values you are talking about. NA can be measured in food, you can measure them in feces, and you can measure them in the urine, etc.

Cohen:

I certainly don't quarrel with the fact that it can be measured, but the issue is to what end, what use can be made of the information once available. Let me, by way of illustrating that, ask you to analyze the *in vivo* acid-base disturbance that I illustrated in my talk in terms of the components of your "titratable acidity" and to reconstruct the sequence of events that led to that situation.

Gambino:

That's just the question that I was going to ask. My analysis of what you said, based on what Dr. Engel said, gives a different interpretation of it. You said your paper described the effects of hypocapnia. But I saw it as a secondary response to using your model to different types of acid load. You had an oral acid load, increase in hydrogen ion load absorbancy, and you had 9 percent oxygen, which I would suspect to be a primary anoxic acid load with lactic acid.

Cohen:

No, Dr. Gambino, there was no lactic acid production in that experiment. That degree of hypoxia produces no increase in lactate or unmeasured anion concentration at all.

Gambino:

How do you explain the bicarbonate?

Cohen:

That was the consequence of the renal response to the hypocapnia; the reduction in bicarbonate reabsorption at the renal level resulted in a steady-state reduction in bicarbonate concentration, unaccompanied by any change in unmeasured anion concentration, no measured lactate accumulation. In other words, the decrement in bicarbonate was offset entirely by an increase in chloride concentration and the change in bicarbonate being accounted for by a reduction, by my terminology, of net acid excretion. There was an accumulated change in acid excretion by the kidney, that led to retention of endogenous acids that reduced bicarbonate to that extent. So what one is seeing in that experiment is the physiologic response of the kidney to two independent variables. One, the oral hydrochloric acid load, and two, the effect of the hypocapnia on renal bicarbonate reabsorption.

Weisberg:

If what you say is true, and I'm not doubting that, if you have that with a normal dog without having the previous hydrochloric acidosis and have the same delta that you had with the decrease in the PCO_2 with the hypocapnia, why shouldn't you have the same effect in the kidney where the loss of the bicarbonate is leading to that acidosis?

Cohen:

We find the same retention of hydrogen ion in the two situations, whether one goes from normal to the chronic hypocapnic state or from the acid-fed to the chronic hypocapnic state. The difference is in the initial level of plasma bicarbonate. In one, it is normal, and in the other it is low due to the acid feeding, but we are superimposing the same delta bicarbonate on these two different baseline levels; as a result, we see the divergent responses of hydrogen ion. There is nothing magic about this. Given the change in PCO_2 and the physiologic response of the organism thereto, the change in acidity is predictable from the relationships described by the Henderson equation.

Siggaard-Andersen:

It should be kept in mind that the same end result is obtained if (1) the initial disturbance is an acute respiratory alkalosis which is subsequently modified by metabolic compensation ending in a chronic respiratory alkalosis, and if (2) a metabolic acidosis is initially induced before the respiratory alkalosis develops.

Cohen:

I certainly agree with that. I don't think the sequence in which these two maneuvers are employed is crucial. One could reverse them and end up in the same situation, I would predict. But we haven't done that experiment. But I still think that the issue is how does one unravel such complex situations. What is the conceptual scheme one utilizes to dissect out the two components which are in that acid-base disturbance; it would be instructive to see how Dr. Engel would do that.

Engel:

Yes, I should first like to draw a parallel to that. We were at one point measuring acid-base balance on patients with pyloric stenosis and, as you know, the interpretation of the concurrent alkalosis has always been that, they developed alkalosis, because they are losing hydrochloric acid in the vomit from the stomach. Now making, for five or six days, balance studies on patients with pyloric stenosis showed that the alkalosis only partly was due to a change in the net acid balance of the patients, and that most of the deviation in Base Excess could be related to the fact that the patient at the same time lost large volumes of water. Thus, it was rather a "contraction" alkalosis. I think that if we want to bring about new ways of assessing acid-base metabolism in practice, and if you want to learn new things, you cannot avoid measurement of external acid-base balances.

Siggaard-Andersen:

One of the problems is that the lactate ion behaves chemically as a neutral substance when we try to titrate it to pH = 7.4. Nevertheless, we prefer to consider it a base from a clinical-physiological point of view. In Engel's terminology, lactate is called a metabolic organic acid (MOA) with a negative sign. I prefer to call it a metabolic organic base, in other words to distinguish between metabolic acids and metabolic bases, in order to avoid talking about a component with a negative sign. The negative sign should not refer to the component but to the measured quantity: the change in the amount of substance of the component in the organism or the body fluid.

I would like to use this opportunity to make a plea for the SI system. It is important that the SI system, which has been advocated quite generally for science and technology, is also adopted by the clinical sciences. It is important that those of us who are involved in the measurement of physical and chemical quantities know the terminology of physics and physical chemistry which constitutes the basis of clinical chemistry and clinical physiology.

However, we cannot expect all physicians to remember all their physics and chemistry, and therefore it is our responsibility to translate the information contained in the clinical-chemical data into common language.

The acid-base data including electrolytes represent a complicated set of data, perhaps even including redundant data (*e.g.*, total CO_2, bicarbonate, base excess, buffer base). Much of this information may be contained in "diagnoses" such as: partly compensated metabolic acidosis, hypokalmeic alkalosis, etc. We may have to make slightly longer statements but anyway this set of terms (acidosis, alkalosis, etc.) is very useful as a supplement to the mere list of measured quantities.

Engel:

I agree completely with Siggaard, and I think that there is one pitfall we should avoid, and this is to let our instruments mix interpretation into the measured quantities or into the derived quantities. I think the manufacturers should stay far away from that. Interpretation is the business of the clinical physiologist or the clinician dealing with the patient.

Bates:

I'd like to ask Dr. Austin a question. Was your pK' a concentration constant or was it the same as Dr. Maas' constants?

Austin:

It was the pK arrived at by determining the pH of whole blood, fixing the PCO_2 by known gas concentrations and measuring the CO_2 content by the Van Slyke manometric technique.

Bates:

In other words, the hydrogen ion is supposedly an activity but the others are partial pressures.

Austin:

Right.

Bates:

They are remarkably constant.

Cohen:

Harkening back to Dr. Bates' first question from this morning, I would like to ask Dr. Maas if he would have any objection to simply taking the negative antilogarithm of the equation which you put up, I've forgotten what the numbers were, I think 16 or 17 or something of that series. In any event, would he be willing to express the hydrogen ion concentration, in quotation marks, as being equivalent to the ratio of PCO_2 and bicarbonate ion concentration multiplied by the negative antilogarithm of your pK value. In this way, simply converting into linear units of activity or concentration, the logarithmic units of pH, in order to specify the level of acidity.

Maas:

You can consider an equilibrium between gaseous CO_2 and bicarbonate directly without looking at the H_2CO_3 in between. So the answer to your question is, yes, the ratio PCO_2 and bicarbonate.

Noonan:

I wonder if we can get some discussion on this whole idea of talking about the plasma pH, plasma PCO_2 versus the similar quantities in whole blood. Does anyone have any feelings about which it should be, or should we have one, or should we always talk about plasma pH in measuring blood pH.

Austin:

The difference is about 0.01.

Weisberg:

The difference is that we now have convenience. The machines usually use whole blood; remember, we used to use plasma. Plasma is wonderful because you never clog the capillary electrodes. When you have whole blood, you have all the problems. But we're lazy. Machines are made that way, so it's easier to put in whole blood. And, of course, it's easier mechanically, but technically, as Dr. Austin pointed out, we're measuring the same thing. So it makes no difference technically. Clinically, that 0.01 difference between plasma pH and whole blood pH is not going to make any difference. And there's no major difference between the PCO_2 in plasma and whole blood as far as clinical purposes are concerned. I don't see you point, but it is interesting.

Gambino:

We don't have thermostated separation systems. So you have serious temperature errors in pH but even more so in oxygen and PCO_2. The other factor will come out tomorrow. These data are utilized by anesthesiologists and ventilation therapists, etc., for the immediate treatment of patients within minutes of having the results. Whole blood is essential. It's not just a matter of being lazy.

We were discussing pH and base and this is a workshop on blood gases and most of my blood measurements are performed because there is a need to know CO_2 gas pressure as a measure of ventilation and the partial pressure of oxygen as a measure of diffusion. These measurements are used to change ventilation therapy. And that's the most common cause for the high volume testing, and the acid-base part of it is important but not as dominant in the minute by minute therapy in the intensive treating. So I think we will have to also consider the fact that there is a need for gas measurements for ventilation therapy.

Laver:

I prefer not to address myself to that problem. Dr. Rispens, to be sure I understood you correctly, did you say that the ratio of the CO_2 content of plasma to that for whole blood was constant over a specific temperature range? Did you measure the CO_2 content of whole blood?

Rispens:

In anaerobically stored blood the ratio of total CO_2 in plasma to total CO_2 in blood is constant. I measured total CO_2 both in plasma and in blood. I emphasized that it was not influenced by the temperature at which centrifugation of the blood took place.

Laver:

Did you say whole blood? In other words, the CO_2 content of plasma plus and red cells?

Rispens:

Yes.

Laver:

So, in other words, the ratio of the CO_2 content in plasma to the CO_2 content of whole blood was constant over a large temperature range.

Rispens:

I emphasized that it was not influenced by the temperature at which centrifugation of the blood took place.

Laver:

Let me start over again because I think it important we do not misunderstand. The patient is at a low body temperature. CO_2 content of whole blood rises more rapidly than the CO_2 content of plasma. If blood is equilibrated at a low temperature, let us say 25 °C, and we now measure the CO_2 content of whole blood and plasma, what happens to the ratio between the two as compared with equilibration at 37 °C?

Rispens:

Well, we measure the pH at 37 °C and put that in the equation [*i.e.*, equation (2) in the paper "Quantitative relationships ..."] to calculate the ratio between total CO_2 in plasma and total CO_2 in blood.

Laver:

Let us assume we measure it by your technique, that of Van Slyke, or with the pH and PCO_2 electrode. I still keep coming back to the plasma versus whole blood CO_2 content.

Austin:

CO_2 content is constant regardless of the temperature. Dr. Severinghaus and Dr. Nunn have shown that.

Laver:

I am sorry to be persistent, but you have not understood my question. If you equilibrate whole blood at a certain PCO_2, for example 40 mm Hg at low temperature (25 °C), either in a patient or in a tonometer, measure the CO_2 content of whole blood as well as the CO_2 content of plasma and compare that to the content for each when blood is equilibrated at 37 °C, what happens to the ratio between the two?

Rispens:

I said that in anaerobically stored blood the ratio between CO_2 in plasma and CO_2 in blood is constant. Thus if you manage to get in the blood the same pH at 37 °C by equilibrating at 37 °C with a higher PCO_2 than you used at 25 °C, the total CO_2 concentration will be the same and so the ratio.

Laver:

Yes, you keep saying total CO_2. Yet CO_2 content in the red cell and CO_2 content in plasma refers to two different things.

Gambino:

You've come back to something reported many years ago, namely, that whole blood with 15 grams of hemoglobin and with a changed temperature, you don't get any change in pH with changing temperature. Let me draw it on the board. This is invariant with the temperature when it is whole blood, but its not invariant with the temperature when it's plasma. I think that's what you're driving at, right? If the temperature changes in a whole blood system, the solubility of CO_2 gas causes changes in one direction and the ionization of the proteins, including hemoglobin, changes in the opposite direction so that the net pH remains constant.

Rispens:

That is certainly true, but that is not the point I wanted to make. I took a blood sample, centrifuged it at 20 °C and 37 °C and found that the ratio of CO_2 in plasma and CO_2 in blood was not affected.

Howorth:

It wasn't entirely clear about the method for measuring total CO_2. Do you always use plasma or do you sometimes use anaerobic blood?

Rispens:

I use blood.

Howorth:

One of the common techniques used in England is to separate the serum or plasma and then measure total CO_2 by acidification extraction using the Technicon AutoAnalyzer. You can get serious losses of CO_2 from the analyzer cups prior to analysis.

Weisberg:

When Skeggs first described the AutoAnalyzer in 1957, that was a fine thing. Then in 1960, he designed the technique for the CO_2 combining power, and, you may recall, he put the entire tray into a box which was equilibrated with CO_2 gas. Two years after that, about 1962, Masters had a different device. Some of you may remember the AutoAnalyzer when they used to do hemoglobins and they added a little stirrer just before it was aspirated, to bring up the hemoglobin. He had a crook for the CO_2 gas going in three times for each cup and so he didn't have to put the entire tray into a box, but they did a CO_2 combining power, and therefore, representative of bicarbonate. Dr. Cohen and I were just talking here quietly and he mentioned Dr. Gambino's technique with the alkalinization. Dr. Gambino and I had many discussions on this. There is no way as far as I'm concerned, by definition, that the AutoAnalyzer can give you a CO_2 content, because it's not anaerobic and you don't have the entire blood as a true plasma specimen. Whether you do the alkalinization or not, it's not going to be, by definition, and that's it. Dr. Whitehead in England showed that very definitely.

Austin:

I think what we're saying is that there is a difference between the CO_2 content of whole blood and plasma. Van Slyke showed this many years ago.

Ladenson:

I think the point is that when most of us measure the CO_2 content by the AutoAnalyzer, we are off by perhaps as much as 3 or 4 milli-equivalents per liter due to CO_2 losses to the atmosphere in these little sample cups.

Weisberg:

You're really getting the bicarbonate.

Gambino:

That's something that Harry Weisberg and I argued about for a long time. I think you're absolutely wrong. In the alkalinization technique, we drive the PCO_2 to about that of the atmosphere and when the CO_2 of the sample is at atmosphere, in a 15 minute period, you don't get any significant exchange. You don't measure bicarbonate. When you lose CO_2, you have a loss of carbonic acid and bicarbonate and you come down to a PCO_2 of about 4 or 5 mmHg. We've done a lot of work in this area and we do 400 or 500 6/60's a day and several hundred blood gases. Many of the patients have the same measurements and physicians are looking at the calculated bicarbonate and the measured bicarbonate, and if they disagree by more than 2 milli-equivalents, we hear about it right away.

Weisberg:

You just said bicarbonate, not total CO_2.

Gambino:

No. We give them a calculated total CO_2, which you can get from the pH and PCO_2.

Austin:

I agree with Dr. Gambino. There is very little difference between the calculated CO_2 content of plasma and the AutoAnalyzer CO_2 content.

Gambino:

When the sample is handled properly.

Cohen:

May I add my signature to that document. I agree. We have done thousands by the Gambino technique and they are extremely close to the Van Slyke...

Weisberg:

You put them into the equation that Dr. Rispens and Dr. Maas were talking about for research purposes, or were you talking clinical?

Gambino:

This in clinical.

Weisberg:

OK. Fine. There's the difference. By definition it is not a CO_2 because it is not completely anaerobic. If we do say that it's a true CO_2 content, we have to change the original definition.

Gambino:

That's a different story.

Weisberg:

That's what I'm talking about.

Gambino:

I'm not recommending it as a primary standard method. If you don't alkalinize, you get disasterous results.

Weisberg:

What do you do with a patient who has a high PCO_2 of 100 and you alkalinize? You're changing it down to room conditions, and you're having to change what was existing in that patient at that time at his temperature. Under most normal circumstances I would agree with you. But when you have extreme changes in PCO_2, high or low in a patient, you're bringing him up or you're bringing him down to those room conditions and, therefore, you're not having the same conditions for your patient.

Rispens:

I hope there is no misunderstanding on our method of measuring total CO_2. There is no escape of CO_2 possible before the sample is added to the acid reagent.

Engel:

I should like to return to Dr. Rispens paper in which he mentioned five reasons not to use base excess or standard bicarbonate. They are not required he says. But what is required? You can use the same five reasons against actual bicarbonate concentration. If you have enough information to define the system you can of course calculate or derive the remaining variables.

Rispens:

After measuring pH and bicarbonate you can, of course, calculate base excess with any nomogram, but what I stress is that you do not need it for assessing disturbances in patients.

Engel:

But we have a very convenient method to determine base excess. Why shouldn't we use it then.

Rispens:

Because you do not need it.

Engel:

But you don't need bicarbonate. I don't need bicarbonate if I measure base excess, of course. Why should I need bicarbonate?

Rispens:

Actually you need it for calculating base excess. Calculating base excess is in fact always based on quantities related to bicarbonate through the Henderson-Hasselbalch equation. Deriving a new quantity from the same measurement results can not produce any information which is not already contained in measured values.

Engel:

If you go to the second reason I would like to say, and so what? This is again the business about the interpretation of results and not measurements of data. An interpretation always has to be done with regard to the patient and the ongoing disease and the history of the patient, etc.

Rispens:

Now that's what I have been saying continuously. My objectives against base excess and standard bicarbonate is that they are presented as a quantitative measure for the metabolic component of an acid-base disturbance. Base excess does not always mean excess of base. In the education of medical students we teach them in the second year how to assess acid-base disturbances from pH, PCO_2 and bicarbonate. We feel that they then clearly understand what is going on. In the fifth year they go to the clinic and, coming back in the afternoons to the laboratory, they appear quite confused by all those derived quantities such as standard bicarbonate and base excess.

Engel:

I can tell you that in our hospital it's exactly the opposite, because we taught them from the very beginning about base excess and they think they do understand base excess.

Weisberg:

Can I alleviate this heated discussion between Ray and myself in the sense of what we use, and I'll have a different track when I talk a little bit later. Mark Twain said, "To do good is noble; to tell others to do good is also noble, but much easier."

Ladenson:

I was very pleased to hear the separation of diagram and nomogram made because I think that the two have completely separate uses. If I understand correctly, you use the diagram as a clinical aid and therefore it has to be evaluated on that basis. But I would submit that the nonogram is very close to a cop-out; because all you're doing with a nomogram is trying to calculate a parameter that you either cannot measure or choose not to measure. In many cases, I think this can be dangerous. In tommorrow's session perhaps we can comment on McLain-Hastings calcium nomogram, but even today Dr. Malenfant commented on a nomogram for oxygen saturation which cannot be used at high levels of carboxyhemoglobin or methemoglobin. In our institution, these are the situations when we often need an oxygen saturation estimate, when we are analyzing the blood of firemen or burn patients. Also we have seen patients following an industrial chemical explosion with severe methemoglobinemia in whom oxygen

saturation measurements would have been useful. This limitation may not be common but I think that what is really needed is a better method which would be accurate under any and all circumstances rather than a nomogram. I think it really is misleading and very dangerous to depend on a nomogram.

Weisberg:

Not quite, Jack. Nomograms are fine because they're based upon data derived in the laboratory. They are not theoretical; they may be based upon a formula based upon facts. But what you're talking about is that they are not taking into account the methemoglobin and carboxyhemoglobin. They don't have nomograms that way. And that's where the mistake is, that you don't realize that you don't have it all as oxyhemoglobin.

Ladenson:

But the problem in that, Harry, is that new compounds have to be continually added to correct the nomogram. I think if you look at some of the current ones you'll see that you have to measure six parameters to get at one. Perhaps we should have been spending the time getting a better method to measure that one.

Weisberg:

Agreed.

Runck:

This question is directed to Dr. Weisberg, Dr. Visser, and Dr. Siggaard-Andersen. Is it possible for the three of you to agree on an equation for base excess? I've heard three different versions of the base excess equation being given.

Weisberg:

I'll agree with Dr. Visser, that you need a big computer to take all these things in. It is impossible to do it otherwise.

Visser:

You see that when you change the pH of blood, there is a water shift from the plasma to the cells. You could call that buffering. Because of the displacement of water from the plasma to the cells when you increase the PCO_2, you decrease the water content, the bicarbonate concentration is increased and so the resulting difference in plasma pH is smaller than you expected. So now you have to define the base excess including the water shift. That makes it extremely complicated. You introduce the Donnan equilibrium, you have to introduce osmotic pressure, you have to explain why the erythrocytes don't burst, and then we need a big computer to do a small job. You may have a practical definition of base excess which is accurate within one unit. That's another thing you can aim at, but if you want to have an exact definition of base excess, an exact calculation, you're trying to shoot a fly with a cannon.

Weisberg:

The other way you're trying to shoot an elephant with a popgun.

Siggaard-Andersen:

Which is the best equation or algorithm for base excess? All equations or nomograms are based on empirical data for titration curves of blood and plasma, or buffer values for hemoglobin and plasma proteins. All approaches involve certain approximations and it is not possible to say generally that one algorithm is better than the other. The reference value will always be the value measured directly by titration.

Cohen:

I would just like to ask Dr. Engel and Dr. Siggaard-Andersen whether or not they would agree with the assertion that I made, that if one is beginning from the vantage point of

wanting to develop a system that adequately described the acid-base status of a living organism, whether or not they believe that there is any less information required in developing such an analysis utilizing the concept of base excess or standard bicarbonate than is necessary utilizing the bicarbonate and PCO_2 itself?

Siggaard-Andersen:

For the evaluation of the patient we actually do not need any of the derived parameters (bicarbonate, standard bicarbonate, base excess, buffer base). All we need is the pH and the PCO_2 and a chart (like the one Dr. Weisberg showed). By plotting pH and PCO_2 in the chart we can evaluate the degree of metabolic acidosis or alkalosis as the distance from the reference line or the reference area for acute respiratory disturbances.

Those who are critical of calculating the excess concentration of base in the extracellular fluid as a measure of a metabolic acid-base disorder, but prefer to estimate the deviation from the reference line in the chart more loosely, are actually deceiving themselves because estimating the distance from the reference line actually involves estimating the excess concentration of base in the extracellular fluid.

Cohen:

I'm sure you understand that I didn't mean to imply that I thought there was anything *less* informative about the base excess or standard bicarbonate. What I'm trying to understand is whether or not your position is that there is *more* information contained in these parameters, *i.e.*, do you require less physiologic understanding of the behavior of the organism in order to unravel a complex situation when using these parameters as opposed to the pH and PCO_2 and/or bicarbonate.

Siggaard-Andersen:

It requires the same amount of physiologic understanding to interpret the base excess value as the interpretation of the bicarbonate. However, there is more relevant information contained in the base excess value as exemplified by the direct proportionality between the change in the extracellular base excess and the rise in the lactate concentration during muscular exercise.

Engel:

Well, I agree with Siggaard. I think that in most cases, maybe 80 percent or more of the cases, it doesn't matter whether you are using one or the other measure, but for certain specific cases, for example the exercise case, I also think that you get more information out of using the base excess than you do using the carbonic acid-bicarbonate system. But we want to go one step further, and think that if you in the exercise experiment had divided the base excess, and preferably the base excess measured at a PCO_2 of zero, up into the net acid part and the metabolizable acid part, then you would have had one further piece of information; namely, that what you were dealing with was an organic acidosis as opposed to a net acid acidosis.

Cohen:

The difficulty, of course, is that when we are in real life, using various systems for analyzing acid-base disturbances, we are presented with an unknown. We don't know that exercise and/or anoxia and/or salicylate intoxication or whatever is at issue or what combination of those factors is at issue. So it seems to me that the burden of the system is whether or not it does, in fact, facilitate our understanding of these clinical disturbances. And in that context, I still remain to be convinced that I know anything more about that individual when I know his "standard bicarbonate" or "base excess" than I already have in my head when I know his bicarbonate and PCO_2. I don't see that my understanding has been improved by those additional parameters. This is an old argument. I know that we've discussed it many times. But it seems to me that if, in fact, there is nothing more that I've learned from these additional parameters, then I have to ask myself: Have I given up anything; is there a price or a risk in using these parameters? I'm not saying that it's not as good. Certainly, one can develop an internally consistent system that describes nature perfectly

well using base excess and standard bicarbonate. There is no quarrel with that. But, the question is, if I'm not gaining anything, am I losing something? And if there is anything lost, then it seems to me that the risk-benefit ratio would argue strongly against its adoption. And I think there is a potent loss or risk. To the extent that the term "base excess" or "standard bicarbonate" implies to the unsophisticated some information *vis-a-vis* diagnosis or therapy, there is a potential for misuse. This is obviously not, as I said in my presentation, an indictment of the system but is something that has to be considered as a liability in introducing such terms. I would submit that this is not an issue when one uses bicarbonate and PCO_2 directly for diagnostic purposes.

Weisberg:

Just one comment in reference to Dr. Cohen. Unfortunately, many people, especially in the United States, think that the M.D. after our name stands for Million Dollars. That is not so, I'll guarantee that on a Bible. But also, on the other hand, I don't think the M.D. should stand for Mentally Deficient. And, unfortunately, we have a problem that we can give all the laboratory data, those of us working in the lab, and if you're on the other side, the practicing clinician, we can give all the laboratory data to the clinician and, if he's not going to use it properly, he's going to have a big problem. Let me give you an example. If we have a patient who has 25 milli-equivalents per liter of keto-acids, calculated, what would your guess be for the diagnosis on an acid-base evaluation on that patient?

Cohen:

I don't have enough information.

Weisberg:

That's exactly the point. Because you find more keto-acids with metabolic alkalosis due to pernicious vomiting then you do with keto-acidosis due to diabetic acidosis. You gave me the answer and we agreed; you need more information which you get from the bedside. You can utilize the laboratory data very well whether you use the base excess concept or the pH-PCO_2 concept. The average clinician is not like that, unfortunately, and therefore that's the difficulty that we, who are servicing that type individual with the laboratory data, have.

Cohen:

I'm not so sure from what you've said, what side of the question you've come down on.

Weisberg:

I'm on the side of the question saying that they agree; all you need is a pH and a PCO_2 presented as numbers to distinguish seven diagnoses of acid-base imbalance. You need a bicarbonate (or total CO_2 content) for two additional ones--to distinguish between a mixed and a respiratory imbalance. The only time I use a base excess (or delta content) is to distinguish between the acute and chronic respiratory conditions. Even with that, I still would have to see the patient to make a definitive diagnosis. I'm not hedging and straddling, but that's a statement of the fact of the case that you cannot make a diagnosis without knowing the condition of the patient.

Engel:

Well, I think I agree very well with Dr. Weisberg, and maybe one of the major problems in understanding acid-base is that most people don't know enough physiology.

Laver:

Well, you remind me of a comment made by Joseph Barcroft 50 years ago, namely that the art of successful advertising consists of saying something which is true but totally irrelevant. At the bedside, nothing categorizes the critically ill patient better than the unsteady state. To make much out of derived values in the unsteady state is a potentially dangerous proposition. Despite my experience with critically ill patients, acid-base balance, and gas exchange, I would not know how to use these numbers, and I would be loathe to react upon them. Although we do not report derived data in our institution, they are available

upon request. I like to think that it has not made much difference in the care of the critically ill patient. What is the value of such interpretation when the derived data, based on hemoglobin concentration, are received at the bedside after the patient has had an infusion of two units of packed red cells and the hemoglobin has increased from 10 to 15 g/100 ml? In exercise, a trained athlete may have a blood lactate concentration of 40 mM at a particular pH and PCO_2, and yet a patient with exactly the same values for lactate, pH, and PCO_2 may be moribund. How do we interpret that? Derivation is important when applied to the steady state. Its relevance to the critically ill, characterized by the unsteady state, is highly questionable.

Gambino:

I have just a few comments. One, regarding plastic syringes versus glass and the data that you showed. We've done some studies in our own lab and find that if the blood gas comes to the laboratory 50 minutes after it's drawn, in my opinion, it's practically useless, and what we do are two things. You'll hear tomorrow that we have peripheral laboratories so that we decrease tremendously this transport problem that blood gases should be measured as close as possible to if not in the site where they are to be used, to eliminate that problem. Secondly, we use ice not so much to prevent change but the abundant ice melts and spills and the person who is given the specimen to carry, sees the melting ice and knows he has to hurry. The third thing we do is that the person who brings the specimen returns the report, because, as mentioned, the actual measurement can be done very rapidly. The specimen comes to a central area and the person who brings the specimen returns the report. That way, the person who sent the specimen to us knows what's happening because he expects that report to come back with the messenger and is less likely to be lost because he knows he must come back.

Regarding the syringes, the dominant reason for measuring PO_2 frequently, is to monitor oxygen therapy so you don't get above 100 mm and below 60 mm, approximately. And most of the specimens that are critical are in that 60 to 100 range and are coming within 15 minutes. In my experience, there is no practical problem with plastic syringes versus glass, if the blood gases are done rapidly. There is a problem with dilution. I am concerned about the amount of heparin in the syringe and too much heparin will introduce PO_2 errors in a sample. We've had problems with glass syringes when the barrels are loose. There is a need for an improved collection system that would be impervious to gas and would not have any gas stored in it and would have a sealed barrel that moves very, very easily. Alkalinization that we perform for the CO_2 content, done routinely, is performed when the Vacutainer arrives, with the stopper on. It is centrifuged sealed, so there is no change in total CO_2 content. As soon as the stopper is removed, that's the alkalinization point right at that moment before any transfer takes place, because as soon as you transfer you lose CO_2, much as you do in pouring a Coke or champagne. You're going to lose CO_2 when you move the specimen. Finally, another unpublished study, done at the Presbyterian Hospital in San Francisco by Dr. Burns and the cardiovascular group there, has shown that the Δ venous-arterial pH and PCO_2 is very small as long as the cardiac index is above three liters. As soon as you start getting decreased cardiac output, you increase that spread and, of course, this is the basic problem with venous samples and capillary samples. In very, very sick patients in shock, the patient with a serious problem that requires frequent blood gas measurements, those are the patients that may give you the largest errors. So I think that for the critically ill patient, we must get that arterial specimen.

Ladenson:

The use of mercury in the syringe is very dangerous as there have been two documented cases of embolism due to its use.

Unidentified:

I think that this was intended to be after the specimen was taken.

Gambino:

Even then it's not needed. We used it a long time ago; mercury in the syringe, but it is dangerous to the laboratory personnel as well. But I think it's been shown that you get adequate mixing by shaking and I think that is the most important. It is essential that the

sample presented to the instrument be mixed whole blood and not have any separation. We do see samples coming into the lab that have been iced and you get extraordinary errors in PO_2 if you don't mix that specimen uniformly. So I think that's a critical aspect of presenting the sample to a blood gas machine.

Ladenson:

What is your median delivery time using your peripheral laboratories?

Gambino:

The peripheral labs are in the intensive care units and there are the four intensive care units in the medical center. There are four peripheral labs, and they service those patients in the intensive care unit. So it is as fast as you get the blood and walk to the machine. I would say the mean time is three, four, or five minutes. Now the mean time to the central laboratory will vary from ten to twenty minutes, because of the elevator and walking time, but never longer than 30 minutes.

Weisberg:

Do you have a special runner going up to get the blood?

Gambino:

No, the bloods are drawn at the site, not by the laboratory.

Weisberg:

You mean the house staff...

Gambino:

Or whoever; the inhalation therapist, the anesthesiologist, the nurse, the nurse practitioner, a variety of people draw the specimen. Because radial artery puncture with a 23 gauge needle and no anesthetic is in many ways easier than drawing venous blood. They do multiple punctures every day. And in the 6 years I've been at Columbia, there has been absolutely no serious morbidity, none, from the radial artery. Not true of the femoral or brachial.

Sørensen:

I should like to comment on those plastic containers because we have started looking at these in the last few months. I feel the problem is not so much the plastic as it is the plunger that causes the problem. Because in the plastic syringe, the plunger friction is much larger than in a glass syringe. If you can avoid this by using radial arterial puncture with small needles and having air bleed in the plunger you get a much better arterial blood sample.

Austin:

I've been an advocate of venous blood gases, but I have seen recently a number of patients where the A-V PCO_2 difference has been quite wide. We followed the instructions religiously, the instrumentation I think has been OK, and I just cannot explain it when a patient has not been in shock, but it just seems to be cropping up in about 5 or 10 percent of the patients where the spread is greater than the 10 mm Hg. I just have no answer for it. It disturbs me and I've searched continuously and I cannot find it.

Burnett:

How do you do tha. determination? Do you draw venous and arterial specimens simultaneously using the same collection techiques?

Austin:

Yes, same collection technique using glass syringes.

Gambino:

These are people at rest?

Austin:

People at rest. Patients in hospitals. Warm patients. I'm on one side, someone's on the other side and we're doing arterial and venous puncture. It doesn't usually change the diagnosis or the clinical evaluation, but the spread of PCO_2 does seem to be wider, and I just have no explanation for it. I wonder if anybody has seen that sort of thing.

Gambino:

I have no recent data since we're doing almost exclusive arterial blood work now.

Runck:

Two comments. First of all, Jack Ladenson commented about the O_2 decay. If we're referring to PO_2 decay in a blood gas analyzer, this, in fact, is probably due to a leak in the system. If the blood has been presented as a properly iced sample, whenever PO_2 decay is observed, it can be corrected by really looking at the fit of the electrodes around the system. Hanson and Neville in Syracuse actually determined this when they put their blood gas analyzer in a great big plastic bag and filled the bag with 100 percent oxygen and, lo and behold, the PO_2 decay suddenly disappeared. The second comment concerns use of plastic versus glass syringes. I would agree with Dr. Gambino, you should not discount the use of plastic syringes. There still are people around using glass syringes who allow the bottom of the syringe to fall out when the syringe is full. I think we have to recognize that not everybody is as proficient as we'd like in terms of handling samples; use of plastic syringes make sample handling easier and more reliable.

Howorth:

I have a comment about Dr. Ladenson's paper. This is in connection with the plastic cover used on the AutoAnalyzer. I understood that the reason one has to have an orange plastic cover is because the bilirubin is extremely labile to fluorescent lighting.

Ladenson:

There are two types of plastic covers, one of which is amber and the other clear.

Howorth:

Well, OK. It's essential to stop bilirubin from disappearing due to photolysis prior to analysis. Then the second comment is about the rate of fall *in vitro* in blood PO_2 in leukemia. I understood that primitive white blood cells utilize anaerobic metabolism to a greater extent than normal white cells.

Ladenson:

I have no evidence of my own. The one study that I have found concerning PO_2 decay and white blood cell count did not distinguish as to the cause of the elevated white cell count.

Gambino:

I think it's important that the plate cover did no good in the AutoAnalyzer. One very important reason, when you use the little disk that was described for the cup, what's happening there, of course, is that the disk is effectively reducing the surface area of the sample exposed. The flow rate or volume of CO_2 escaping per unit of time from a particular surface area is decreased. But when you have the cup, like this, you have a big plastic thing, there are all kinds of cracks in it. It doesn't mean a thing, and so the surface area exposed to the atmosphere is the same as it is right out in the open room. This does absolutely no good at all. That's why we never reported on it. It's absolutely useless.

Now as far as the bilirubin is concerned, it is equally useless. If you come to my lab you'll see that we have a stainless steel cover that's opaque to light because the bilirubin will deteriorate through that semi-colored cover. So if you want to get the light effect out, we use a metal cover over the AutoAnalyzer tray, and it is an important factor if you have bright lights or sunshine.

Weisberg:

That kind of cover must be used on the AutoAnalyzer. However, if you allow the cups to rotate for an hour, you will find that if you have it uncovered, the dehydration of the proteins will be increased by about 8 percent. So that's an absolute technique for getting to the one with the acid-base blood gases.

Gambino:

That's not as effective as it should be either, because you still have the leaks.

Weisberg:

Yes, but it's going to reduce it. Without it you're going to have an 8 percent change.

Siggaard-Andersen:

The papers by Dr. Gambino and Dr. Laver were very interesting, but it occurred to me that neither of them stated directly that another worthwhile effort might be to try to reduce the number of requests. I think Dr. Laver hinted yesterday that we produce all these numbers but what do the doctors do with them? There were a number of redundancies among the tests you mentioned, *e.g.*, serum osmolality and serum sodium.

Gambino:

I think your point is well made, I'm very aware of it, and I believe it can be approached in several ways. First, the peripheralization of the laboratory as I conceive of it and as Dr. Laver was suggesting, when the physician himself or the people taking care of the patients actually do the measurements, there is direct control. You see, the laboratory is simply an extension of physical diagnosis. It's a more refined physical diagnosis. Now, the physician, himself, when he performs a physical exam, knows he has only 24 hours in a day and then he has so much time to give to the patient, so he rations his time accordingly. When you have a large central laboratory, and all you have to do is write an order on a chart, that is conceived of as an infinite resource, much as air and light and water. There's plenty of air and plenty of water, but we know it's a finite resource, and a laboratory is a finite resource. So, one of the ways of avoiding abuse of this finite resource is to put the resource directly into the hands of the person who is using it so that he, himself, experiences that. And that's what happens with the peripheral unit. In other words, if they start doing more blood gases, they'll just buy the blood gas machine, set it by the patient, so that providing laboratory services at the bedside is one way and having the person who is utilizing the lab tests become more active as part of that, either by actually doing it or by actually bringing it to the laboratory. In the case of stats in our hospital, a telephone call is required and a stat voucher is made. We do not pay any attention to written stats. There must be a phone call by the physician, it must be an action so that there is involvement. I mentioned yesterday about the specimen being brought to the laboratory. When it comes to the central lab, the person who brings the specimen must wait to bring the report back. That closes this chain. And so we are very, very concerned about it. The second thing we do to eliminate tests, and we just last month no longer do single SGOT's as isolated tests. We don't do HBDH. When we introduce a new test, we always look to see if we can eliminate another test. So just as the physician only has an hour or a half an hour--or whatever the time--to do his physical exam, I only have a certain amount of time and resource and total energy in the laboratory to expend on these results, so I must monitor what's happening and how useful it is. But I agree that it is easy for this to take off and be abused. But when the person is doing it himself, it is much less likely. So that the second thing is that I don't think there is going to be any slackening in the lab work because the lab is such an essential component of physical diagnosis. You don't want to wait until the patient is blue to diagnose anoxia. And we don't want to wait until the patient has

cardiac arrest to diagnose digoxin toxicity. Therefore, the physician requires these techniques and, as I told Dr. Laver last night, if you deny a physician laboratory tests, you're effectively denying him access to his patient. It's as if you locked the door on the room and you denied access to the patient. I think this is extremely important for the clinical chemist to appreciate and the people at NBS and the regulatory agencies to realize, that laboratory testing is such a direct continuance from physical diagnosis that you are interacting very directly with physicians' activities. And the physician is not as dumb as sometimes has been alluded to--that they don't know what they're doing. They, indeed, have a pretty good idea of what's happening, and I think that the key thing is for laboratories to provide those tests that are physiologically and biochemically correct. When we understand the physiology and we understand the biochemistry of the disease process, then the testing becomes important. You will find that as our knowledge of any disease process increases, testing volume will increase because the test becomes critical. Now that we understand pulmonary physiology better, the tests are extremely critical. When we don't understand a disease, such as diabetes, testing is very vague and diffuse and not very useful.

Austin:

I think maybe this has been referred to, but it's my contention that pieces of equipment need a mother, so to speak, and we have found that when errors develop and when deviations occur it's because too many people have access to a piece of equipment. They really need to be, in my opinion, supervised by a very few people and I think when you get too many people using it, too many handling it, that's where it breaks down. That's my theory.

Laver:

In regard to Dr. Siggaard-Andersen's comments--yes, you are quite right about osmolarity. We introduced it a long time ago when we did not have immediate access to a blood sugar measurement and, of course, the blood urea nitrogen. Now, we receive few requests for serum osmolarity. However, matters are quite different when one is dealing with blood gases. These patients are usually in the early phase of acute respiratory failure. It was our original intent to provide continuous measurements by repeated analyses and a short turnaround time. Depending on severity of disease and the number of patients in need of ventilator support, we perform an average of 400 blood gas determinations daily in some 80 patients. Fortunately, the number of patients in need of our services has not increased, and the average number of samples per patient has not changed. The overall demand is not likely to be altered until we have reliable, continuous blood gas measurement devices.

Richards:

I direct this to Dr. Gambino. One of the figures I jotted down was a relative standard deviation of 11.3 prcent for PO_2. If you took that at the 2 standard deviation level, it would mean about 22 percent.

Gambino:

Yes. It's not tolerable.

Richards:

It seems to me, if this kind of quality figure is being generated, it might be better for a laboratory to cut back on the work in order to provide a better quality statement. For those of us who participate in proficiency testing, 22 percent is really very high. This is the sort of thing you see for some of the enzymes.

Gambino:

I didn't want to give the impression that I found that acceptable. I'm telling you that this is what was seen when a particular study was performed in different laboratories. In other words, across laboratories, and of course you have to realize that indicates that there are problems with instruments in the field.

Kreuzer:

I would like to return to Dr. Laver's remark. It is also my contention that the future is continuous recording, and I would like to draw the attention again to the group in Brussels which is led by Dr. De Meester. This group has embarked on a rather large program to apply these continuous recordings. Of course, I am well aware of all the problems, but I think it's worth the effort. My second remark also refers to Dr. Laver's paper. I wonder whether anybody has had experience with the microporous membranes as applied to measuring problems *e.g.*, in polarography, because microporous membranes might be just the material we are looking for. This is due to the fact that we are concerned here with a suspended gas phase in essense; a large part of the membrane is essentially gas phase. You have high diffusivity and practically no solubility. Has anybody had experience with these membranes?

Brand:

I tried using such a membrane. You refer I take it, to something like the microporous Teflon membrane that Millipore makes, that kind of material. In our experience, it clogs with red cells very quickly. It works very well for about half an hour and then clogs up irreversibly.

Engel:

I would like to return to the problem of the decentralized laboratories and I agree with Siggaard that when you get up to a size as we have in our laboratory, with more than 200 employees, it is comparable to a power station where the requests come in just like when you turn on a switch. But I also agree with Dr. Gambino that establishment of peripheral laboratories probably will reduce the number of tests which are not really necessary in order to handle the patient. We have a very good example, when we made a peripheral laboratory in our pediatric department, the number of tests from that department went down by more than 50 percent.

Visser:

I think that the need for blood gas analysis can be enormously decreased by using continuous CO_2 monitoring in the expired air. In Europe, this has been in use for about 20 years. Twenty years ago I had to build my own infrared analyzer because I had no money to buy one. It is now everywhere in use, hundreds of analyzers, and especially for respirator treatment of patients with normal lungs, you don't need the blood gas analysis because, in that case, it is especially the CO_2 and the pH that is of importance. And the O_2 doesn't change so rapidly. So this is also decentralized, and you can do it as continuous monitoring or intermittently, if you don't want to buy so many instruments. Of course, you can't do that for oxygen. There is a big difference between Europe and the United States, in that respect.

Laver:

I think that comment requires a vigorous rebuttal, particularly from someone whose experience has indicated that end tidal PCO_2 measurements are worthless in the critically ill. First, because the difference between end tidal and arterial PCO_2 can be substantial. Second, the blood-gas exchange problem in patients with acute respiratory failure (ARF) is oxygenation, not CO_2 removal. The latter is apparent as a terminal event in ARF and common with patients in chronic respiratory failure. To my knowledge, there appears to be little difference of opinion on the subject. Monitoring of end-tidal CO_2 may be of value to follow performance of the ventilator but not for evaluation of blood-gas exchange in the patient with acute respiratory failure and previously normal lungs. I find no justification to condone this return to a questionable practice.

Visser:

That is not what I am proposing. I am proposing a decrease in the number of blood gas analyses by replacing a number of blood gas analyses by measurement of alveolar or end tidal CO_2, taking into account the difference between end tidal CO_2 and arterial CO_2. If you have a patient's arterial and alveolar PCO_2, you can follow changes of PCO_2 by measuring expired gas instead of blood. So the number of blood gas analyses is decreased but not replaced by ...

Laver:

Unfortunately, things do not work out that way in practice. The number of blood gases is not reduced because one must still follow the level of arterial oxygenation. Occasionally, samples must be drawn every 10 to 20 minutes because repeated evaluation of arterial PO_2 is necessary. However, such determinations include the PCO_2 and pH. Addition of acid-base evaluation does not complicate or lengthen the procedure by more than a few seconds.

Kreuzer:

I'm inclined to agree with Dr. Laver, and I might add that the electrodes which we have described are equally applicable to the gas phase, to the continuous recording of PO_2 in the respiratory gas. We have done hundreds, even thousands of experiments over many hours, with recordings in all kinds of situations. We have even developed a telemetric method. I might add here, since I couldn't go into this for lack of time, that we succeeded to telemeter, over distances of one to two kilometers, the oxygen uptake of a subject by means of, among other parameters, the continuous recording of PO_2 in the respiratory air.

Siggaard-Andersen:

Has anybody had experience with the use of the isotonic bridge described by Maas some years ago? The isotonic bridge eliminates the effect of varying hemoglobin concentration on the liquid-junction potential but it does not eliminate the effect of varying ionic strength in the sample. The latter effect can possibly be calculated by means of the Henderson equation. In dehydrated or over-hydrated patients the ionic strength of the plasma may vary considerably which might cause a considerable variation in liquid-junction potential. This would be of special importance with the calcium electrode where the electrode sensitivity is only half of that for a monovalent ion.

Austin:

I'm not sure I'm answering your question, but I think saturated liquid-junctions give more stable readings in my experience. I'm not sure that's what you're asking, but that's my experience.

Bates:

I'd just like to point out, in connection with that figure of Dr. Maas, that strictly speaking, these standards, which have an ionic strength of 0.16, are not on the NBS scale exactly because the NBS convention was limited to 0.1 ionic strength. However, I think the excellent agreement that you find experimently might be justification for relaxing this restriction.

Weisberg:

May I change this subject before Dr. Laver leaves and I'd like to have this on the record. Dr. Kreuzer could answer this too, because back in 1971, Severinghaus and his group reported an error on the oxygen electrode due to the reduction of the halogenated hydrocarbons, especially halothane. This has been in the literature and I've asked several anesthesiologists and it seems to be a puzzle. I've discussed this with Dr. Laver but I'd like him to put it in his own words for the record and perhaps Dr. Kreuzer could answer too. Does halothane have an effect on the polarographic electrode for oxygen?

Laver:

The answer is no. We have run polarograms with whole blood in equilibrium with up to 4 percent halothane, a variety of electrodes, a variety of membranes and buffers (pH 7 and pH 10). We have found no discrepancy attributable to halothane. This holds true for methoxyflurane, fluoxene and other halogenated anesthetics, irrespective of oxygen concentration. The effect of halogenated anesthetics on the readings of the PO_2 electrode need not be of concern to clinical laboratories.

Kreuzer:

First of all, I would like to confirm this, but maybe one word about CO_2 should be added. You keep reading statements in the literature about CO_2 sensitivity of the electrode. We have investigated this very thoroughly, too, and in our experience, if an electrode is markedly CO_2 sensitive, then it is apt to be a bad electrode. If the electrode is constructed properly, (*i.e.*, if there is sufficiently fast exchange of ions between the electrolyte covering the cathode and the bulk of the solution) and if the bias voltage is chosen correctly it is practically not sensitive to CO_2.

Austin:

Just getting back for a moment to the liquid-junction subject, I would be interested in Dr. Bates' feelings about the molarity of the solution for a liquid-junction.

Bates;

Well, I really don't have any special information beyond that presented by Dr. Maas. I think everyone realizes that the early work indicated that, in systems which are homogeneous, the stronger the concentration of KCl the more reduction in the liquid-junction potential that ensues. But I think that with a system such as blood where you have the danger of crenation of blood cells and precipitation of proteins, etc., you may have a special problem. Perhaps that outweighs the matter of the actual magnitude of the liquid-junction potential that you would expect in completely homogeneous systems.

Runck:

I have a comment dealing with Dr. Gambino's statement on the performance of the Versatol Controls and Dr. Noonan's statement on the Coke machine concept. I think Dr. Gambino's data on the Versatol Controls presented two aspects of performance. The first aspect is the precision, or repeatability, of replicate measurements. The second aspect wasn't mentioned and that is the accuracy, *i.e.*, the bias, seen by the laboratories reporting the values. Our experience has been that the precision problem is actually an operator problem. And that can be corrected very quickly by going in and retraining people, showing them how to do it properly. The bias problem is more of a systematic problem, and that has to be looked at in instrumentation. Now the Coke machine type instruments, the automated instruments, tend to deal with the precision problem very well. We find very good precision performance with automated type instruments.

Ladenson:

My question is actually in the form of a request to Dr. Durst and the Bureau of Standards. I hope that future certified pH standards would be checked with systems that have liquid-junctions. For example, when we analyzed the certified Tris buffer against the certified phosphate buffers we got a value that was 0.01 to 0.02 lower than the certified value (*Clin. Chem.*, 20, 1337, 1974). This means that the two certified buffers will give different results on the type of equipment that is used in virtually all laboratories.

Durst:

Preliminary studies made at NBS several years ago indicated that this a real effect, but the magnitude of the problem is still under study. Unfortunately, we do not have clinical pH instruments available for our testing and must rely on classical liquid-junction measurements.

Bates:

Didn't you designate that as a secondary standard?

Durst:

It was supposed to have been issued as a provisional standard until such time as we demonstrated consistency with the pH scale for cells with liquid-junctions. But at the

point where we certified it, the Office of Standard Reference Materials decided that they weren't going to issue provisional standards anymore, and it went out as a final certificate, without any indication that it was to be considered a secondary standard. It was an internal communications problem in that respect. It went out essentially as a primary standard, when it really was provisional as far as we were concerned. The certificate has since been revised to reflect these considerations and also carries the secondary-standard designation.

Kreuzer:

I would like to make two remarks with particular reference to the PO_2 measurement; one, the gas-liquid factor and two, the tonometer. As far as the first point goes, what I often miss in these graphs relating gas PO_2 to fluid or blood PO_2 is the exact description of the apparatus. In other words, the blood liquid-gas factor is nothing else than a stirring factor or flow factor, or a flow dependency as we call it in our continuous system, and it has only meaning if we know exactly what the electrode is, that is to say, if we know the diameter of the cathode as well as the thickness and the material of the membrane. And this is not always self-evident. It may be self-evident for the user, but not for the listener. This factor may be quite different depending on the type of electrode. Radiometer Company, *e.g.*, has used different types of electrodes over the years with different diameters of the cathode. Concerning the second point, the tonometer, it has been described already decades ago that in tonometers there is apt to be a separation between plasma and erythrocytes. If you draw a sample from the tonometer you may easily have blood that has a higher hematocrit than native blood and this, of course, as Dr. Runck showed in one of his graphs, will affect the gas-liquid factor. That is to say, you have to know when you are tonometering what the situation is in terms of the hematocrit.

Gambino:

I wanted to ask Dr. Maas about his laboratory. You presented data on various instruments in your laboratory in regard to your aqueous quality control calibration system. Which junction do you use in routine practice? Do you use your sodium chloride junction or, when you use the instruments, do you use the junction material recommended by the manufacturer?

Maas:

We have used the junction recommended by the manufacturer.

Gambino:

So that you are not in actual practice, in your hospital, carrying out on patient samples, the isotonic junction?

Maas:

No, we don't use that type.

Gambino:

Do you think you will? In other words, we would like to know, to help us to decide which way to go.

Maas:

I feel that in practice by changes of the concentration of the isotonic solutions, for example, by diffusion of phosphate, poorer results are obtained than using saturated KCl.

Gambino:

So, at the present time, you would not favor recommending moving in that direction. That clarifies it then.

Siggaard-Andersen:

Concerning reference methods, I think it might be possible to develop a reference method for blood pH on the basis of the operational definition given by IUPAC, using a capillary electrode, standardized saturated KCl liquid-junction, etc.

Concerning PCO_2 and PO_2, the reference method would probably have to be based on gasometric analysis of a small gas bubble in equilibrium with the blood, *i.e.*, the Scholander technique. This techinque was too complicated as a routine method but with new techniques it may prove useful as a reference method.

Ladenson:

There have been some statements made that this reference material would have to be blood. If it were blood, how could we standardize its use? I cannot see any way of doing this because everyone will tonometer it differently. Are we going to have a standard tonometer? From a practical standpoint, the problem is that all instruments do not give the same results. To those of us in the field, the problem is which one is correct, and how can we ascertain it. To the manufacturer, I am sure the problem is similar when it comes to instrument design. The aqueous material (General Diagnostics) described previously, apparently parallels the differences one sees with blood. This material which was apparently shipped all over the country under all kinds of conditions has very similar results in various laboratories. It appears like we now have a good start on a reference material and we need a central unit to provide values for it. I think we are now sitting in the place that can do this better than anyone and can do it independent of any particular manufacturer's electrodes. I believe the National Bureau of Standards should play a major role in ascertaining whether there is a material that is stable and whether values for pH and blood gases can be assigned to it based on rational measurements independent of a particular commercial instrument.

Durst:

I would like to respond to that briefly insofar as we are being put on the spot. This was one of the purposes, as far as I was concerned, of the workshop; to try to get ideas of what could be used as a good standard material for calibrating pH and blood gases. We were thinking along the lines of one of our buffers in isotonic saline and coming up independently with a gas mixture of certified CO_2 and O_2 with the balance in nitrogen. Then one would have to develop a reference tonometer to put them together and come up with a reference solution. But it seems from the discussion of the materials that Dr. Gambino described that there may be a material already available that we could use in this way.

Gambino:

I want to clarify that. I think it's essential that the National Bureau of Standards provide a reference material for gases, in other words, a certified CO_2-O_2-N_2 mixture as a reference sample. So I think that's step number one. That can be done. Your mass spec can help you there. You've got the technology to do that. That will fix a reference point. Now then I think the material such as I showed, and the material that Dr. Maas has developed in Holland, are secondary materials and will be dependent upon the primary gas standards. If you don't go to blood, you can define the PO_2 of those systems, and you can also measure, you can recover, you can do gas chromotography, you can analyze that solution which is a simple solution, a single aqueous sterile solution, and quantitate the amount of gas. And from the solubility coefficient, etc., you'll know what PO_2 to expect. But I would see it as kind of a secondary reference material, not something that the NBS could successfully do immediately, or should do. I think you should definitely do the gas as soon as possible.

Austin:

The same thing should hold true for buffers.

Gambino:

Well, they've done that. Along those lines, I think the NBS should consider doing more work at 37 °C on other buffers. I think that would be essential for more physiologic research.

Weisberg:

What about bracketing the normal pH with something above 7.4?

Gambino:

Exactly, something higher, a buffer certified at 37 degrees that would go above 7.4.

Rispens:

I think as far as PCO_2 is concerned, it is much easier. You do not need gases. You can make a mixture of a bicarbonate solution and a phosphate buffer which has a definite PCO_2 after mixing.

Gambino:

Do you supply gases for any other type of standard?

Durst:

Yes, for example, we have certified air-pollution gases which are mixtures of CO_2 and nitrogen.

Runck:

Blood gas measurements shouldn't be any different than any other clinical chemistry measurement. We need both a standard reference material, as suggested by Dr. Noonan, which is traceable to NBS, but in addition, we must have a standard reference method for the determination of blood gases, just as we have atomic absorption for calcium and hexokinase for glucose.

Ladenson:

The only problem I see in stopping there, is that some manufacturers have designed instruments that would not be compatible in routine use with a primary gas standard, *e.g.*, the Radiometer ABL1 system. For this reason, I believe that we need something that will be applicable to all instruments. I wonder, and I address this to the manufacturers, would such a non-blood material be acceptable to you as a starting point to try to get these values together.

Runck:

Absolutely.

Ladenson:

Then I think that is what has to be done.

Austin:

Everything is relevant to that.

Ladenson:

Well, we can all agree that with such an NBS certified material we can begin to standardize the results obtained with different instruments.

Gambino:

It would be possible with an NBS gas in a defined tonometer system to reproduce ...

Ladenson:

What I'm saying is that the material has to be made or purchased in large single lots in the same way as all the other Standard Reference Materials. NBS writes the specifications, analyzes the material, and provides a certified value for a material which you can ship around the world.

Unidentified:

I don't know how other people feel about this, but I found using a wide variety of different types of tonometers, made in different ways, that it's not that difficult to tonometer a solution accurately.

Runck:

That depends, in our experience, on the level to which you tonometer your blood samples. Most of the data we have seen are at normal values for PCO_2 and PO_2. Those don't stress the instrument. It would probably be advisable to have, in addition to normal ranges for PCO_2 and PO_2, a control material or a reference material at the extreme values as well.

Gambino:

I would disagree with you, at least clinically, maybe in physiologic research you might want it, but clinically we don't want patients to go above 110 millimeters at all.

Weisberg:

What if you have a patient on therapy?

Gambino:

That is why we do so many oxygen tension measurements and nobody is supposed to go over 100 millimeters, period. That's why we do it. You keep lowering the inspired oxygen and that's why, in my particular laboratory, I couldn't care about accuracy at very high PO_2's.

Weisberg:

You get levels of 300 and 400 in cardiac bypass.

Gambino:

So what? It doesn't make any difference.

Weisberg:

The point being that they want those with accuracy, and they're trying to make a decision on it.

Gambino:

No they don't. They don't want it at all.

Runck:

I think there's controversy on that. There are two points on the table right now. Number one, can you tonometer accurately? Number two, if you can, what are the correct levels to use? Each has to be treated separately.

Gambino:

There may be some special physiologic situations in a hyperbaric situation where somebody's trying to maintain a high PO_2. In a hypothermic situation, they want a high amount of oxygen physically dissolved. Those are special cases. I think when you see the problems

that are occurring, however, the critical decision levels are at 100 and at 60. Those are the dominant decision levels. It's essential that we be accurate and precise at those levels. I think you get into tremendous sample handling problems with a high PO_2. At high PO_2, you have to go right from your tonometer or your patient directly into the instrument. If you have any type of sampling handling, you're not going to get the right value.

Siggaard-Andersen:

Just a comment on the technical problems of tonometry. If you have very small bubbles, the pressure inside the bubbles is higher than atmospheric pressure. If you have a very tiny outlet from the tonometer, you may also have a higher pressure. It is not always easy to obtain exactly the same temperature in the tonometer as in the electrode, so if we want accuracies of 0.1 mmHg it is not going to be easy.

Runck:

My comment relates to Dr. Gambino's comment on the levels of PCO_2 and PO_2 at which the operator should test the instrument. I think we must keep in mind that there are two purposes in testing an instrument. First, is to assure yourself that patient treatment is being carried out on good numbers. Second, is to give the operator early warning of impending instrument problems. In the case of CO_2 measurements, if we choose 35 millimeters as our checkpoint for CO_2, the carbon dioxide electrode could actually turn to stone and you would still get very nice readings at 35 millimeters because the electrode is always kept in the area of 35 millimeters. It is very important that a CO_2 level other than 35 millimeters be tested. And the same thing with PO_2. The early warning of a PO_2 problem with an instrument is going to be very apparent at a high PO_2. If you have excessive atmospheric contamination or a leak in the sample chamber, giving relatively small problems at 86 or 90 millimeters, you will see this in exaggerated fashion at 200 or 300 millimeters. This is why manufacturers should state performance characteristics at these levels as well.

Gambino:

I agree with that. You also see it if it is very low.

Cali:

Would it be interesting to the group to find out how we would handle the question of reference materials here within the Bureau, in order to arrive at a reference material?

Group:

Yes.

Cali:

I've heard you talking about accuracy and precision. Where it is possible, we believe that all standardization should be based upon accuracy. I won't go into the complete logic of that proposition but if you are trying to achieve compatability in measurement and by that I mean simply that everyone measuring the same sample gets the same result, then that goal can be achieved directly if all concerned are making measurements that are accurate. So, we start off by asking what is the basis for achieving accuracy? Well, accuracy is related to the true value, but let's not get into a philosophical fight on what we mean by "true value." But you can say in an operational sense, that somehow you've got to have access to the base and derived units of measurement, which is now the SI system. We have access to the SI by what we call here at NBS definitive methods. Now, definitive methods do not exist, in which case as an alternate, you then use two or more reliable methods. What constitutes a reliable method is a matter of expert subjective and, hopefully, objective opinion. The preferred way, however, is via the definitive method. A definitive method must fulfill several criteria. You must have a well-established theory and usually it should be expressable in mathematical terms, usually via appropriate equations. When you make such a definitive measurement, using the equation, the derived end result is expressed in terms of one or more measurement parameters. Call them A, B, C, and so on. You must then make your measurements in such a way that each of these parameters, A, let's say is

mass, has to be directly traceable, in a well defined series of steps, to the base system in units. And similarly, if B is temperature, you have to know what systematic errors are involved in the making of that measurement, and so on. When you are finally done, you can then say that your measurement is within some error bounds in terms of accuracy, or inaccuracy, to be more correct. Using such a proven definitive method, you are now in a position to take a material and to build into that material the accurate values of whatever property or properties are under consideration. From this we arrive at what we call here at NBS a Standard Reference Material. Now the rest of the world does not like this word "standard", in the phrase "Standard Reference Material". It's a word very difficult to translate into other languages. The accepted term now would be reference materials, in general, and what we make at NBS would be called *certified reference materials*. So we've got certified reference materials (CRM's) and, more generally, reference materials. At this point, because CRM's are directly related to the SI by a one-step process, we call these primary reference materials. Now, this methodology is usually very difficult to apply in the typical clinical laboratory situation. For example, we may use such techniques as isotope-dilution mass spectrometry which involves instrumentation running well over $150,000 or $200,000 plus very highly trained technicians and scientists and so on. Therefore, we want to transfer this accuracy in some way that is of a more practical nature. So now, we go from definitive methods via the CRM to reference methods, which is what you've been discussing. Given then a CRM and a reference method we now have the possibility of the manufacturing community producing accurately known secondary materials, in a matrix that is really of interest. Such secondary materials can be used for two purposes: (1) quality control on a daily basis through these secondary materials, and (2) through the use the reference method and the primary reference material to check in turn the accuracy of what I call the field or routine methods used on a daily basis. This whole process has, of course, variations, depending upon the particular scientific or technological field. In clinical chemistry, I have just described the logical way to proceed toward achieving measurement compatibility. This system is, however, just in its initial stages. Many more definitive methods, reference methods, and CRM's need to be developed before major advances can come about.

Have I heard correctly that, although you may not all be in exact agreement, what you would like from NBS would be a series of gases at the appropriate partial pressures of oxygen and carbon dioxide in nitrogen as a first CRM. As a second CRM, perhaps a buffer that's equilibrated with appropriate partial pressures of those two gases, and then preferably, blood or blood-like matrices prepared the same way.

Engel:

Right.

Cali:

Have I got it about right?

Several voices:

Yes.

Cali:

You'll have it tomorrow!

Ladenson:

I'd like to ask Dr. Bates a question. You said you used Tris buffer to control pH. Did you do any studies with Simon's calcium exchanger on the effect of Tris buffer? Both the Orion and the Ruzicka exchangers will show apparent calcium binding to Tris buffer.

Bates:

We used Simon calcium exchanger, and our studies suggested that there is no significant association between calcium and Tris buffer.

Ladenson:

It would be interesting to know whether the apparent Ca-Tris binding was an artif real because there is no pH displacement even though the electrode indicates a linear relationship.

Bates:

We did, of course, find the linear relationship between the concentration of calc but it's possible that there's an interaction that is responsible for the slope being than theoretical.

Noonan:

I got involved in this particular area a couple of years ago. I haven't really anything since then, but I think some of the points that were brought up today are re quite revealing to me. When I was at Corning, we did some rather extensive studies i direct undiluted measurement of sodium, potassium, and chloride, and the way we had c of doing it was just as you said. We established an activity-concentration factor an compared our results to the results of conventional systems which measure the total s total potassium, and chloride. While on the average we got excellent correlation, th were many rather large differences between what we observed by using this technique a what had resulted from more conventional techniques. I would submit that quantitati results are obtainable from these activity measurements and whether or not you want t correlate them to some normal ratio between activity and concentration is a question convenience. I think these measurements might very well represent the more significa physiological status of the system. One of the things we saw often was chloride val were tremendously influenced by albumin levels. These results would look like norma levels by a total chloride measurement. Looking at them with these electrodes showed icant deviations from normality. And likewise variations in total protein also woul to make electrolytes look differently, since they would directly affect the "average activity coefficient.

Cohen:

How do you interpret that?

Gambino:

Yes, what could a physician do with that data? I think it's important to cons activity and the electrode measurements, but I don't think enough work has been don

Noonan:

When you look at the total, the correlation is excellent. So that you could s we're going to give you 140 on electrode and 140 on your flame photometer, but once while that electrode is going to give you 130, or its going to give you 150. The c studies have just never been done to demonstrate the usefulness of this approach. does seem to me that the physiological activity of these ions relating to the therm of ion movement across cells, etc., are basically a function of activity, and not c tion.

Weisberg:

Seligson did that about 10 or 12 years ago with Dahms and Rock, and they had publication in *Clinical Chemistry* but it's just hanging in limbo because you can't your number to concentration. As Ray pointed out, the clinician is thinking in co for electrolytes, and therefore, it may be a very good method, but you have now go factor to correlate it to the concentration.

Cohen:

The issue is whether or not these are really telling us something that is go the patient.

is old data suggesting that chloride is measureably bound to albumin.

hat is true. One of the things that was missing from my model, of course, was actions with albumin. I specifically left out chloride because I don't believe gnificant binding at physiological pH. If you look back, Dahms used chloride explain some anomalous chloride results that he got. If you look back to the rk of Scatchard, and it's been confirmed on a couple of occasions, then it shows loride binding of albumin is markedly pH dependent, and at physiological pH, it's igible. So I think that there is little justification for including chloride- ding. In terms of CO_2, of course, there's plasma carbamates which ought to have ed in the model that I presented, but I just didn't because the data wasn't readily o me.

k about chloride binding in the physiologic range, what magnitude are you ?

s far as I can remember, the thing that's reported is number of ions of chloride nd per molecule of albumin, and the number in the physiological range is less here is less than one chloride ion bound per molecule of albumin. When you get of around three (or perhaps five, I don't remember exactly) it goes up to about ions per albumin molecule.

ersen:

the report by Dr. Brand very interesting but I wonder how you have estimated the the protein polyelectrolytes on the ionic strength? Just taking the net protein tration and assuming the influence equivalent to monovalent ions is questionable. ated the ionic strength of normal plasma on the basis of the pK value for carbonic ma as compared to pure aqueous solutions and found a value of about 0.17 mol/kg. you have any comments to that.

he ionic strengths that I show are excluding protein. They are electrolyte ionic In other words, I am following the convention of Scatchard, that the activities tes in protein solutions are unaffected by the proteins. Now I don't think that's experimentally by anyone other than Scatchard, so I can't comment, but that's a ritative source. The model also, I should perhaps have commented, does not force lity. It does not require that the system is in electroneutrality and it simply the excess charge, whichever way, is made up by the protein. I think a model loped forcing electroneutrality. It would be an interesting thing to do but I it, and it wasn't included in the model presented.

uggested that, as a final item, we have summary made of what's happened over days, and I didn't want to do this because I am very subjective about the con- disqualified myself. Since I wanted to get a disinterested person, I asked g to provide us with a summary.

e really disinterested, I wouldn't have come. But there's an old Army saying, nited States and I am sure in the army of every country, "never volunteer." I Dick a little earlier asking if a summary would be given and you've heard his why he isn't giving it. I had suggested some names and some of those named t reverted back to me again, you see; so here I am.

I apologize because if I had known I was going to do this, I would have listened mu better to you people. If I leave anyone's name out, please accept my apologies, and I a ask you please to contribute until the time of your leaving for the airport, if I have m any errors or if you object to what I say. I will honestly try to be objective and leav out my personal bias. I have to tell you what Dr. Laver said yesterday when I was kidd him, having known him for some years, why he didn't contribute as much as I did by inter ting, and he said he learned his lesson. He was at a conference several months ago and spoke so much that he now has 15 pages to proof on his added comments; so I am stuck aga on that.

I think that we can have some subheadings for Dr. Rubin and the idea for the Expert Panel. Number one would be the groups of the Definition of Quantities and Concepts to b combined with the Recommendation of Nomenclature and Physiological Terminology and Symbo As mentioned earlier, there is a phoenix that has arisen from 1964, and obviously we sti have the same problems we had at that time. We have the problem of overlapping laborato and clinical terminology. We have the problem of which parameters to use in reference t the diagnosis of acid-base imbalance. Here, of course, I'm mentioning names. Dr. Cohen introduced some additional terms regarding the duration and compensation of acid-base disturbances, and Dr. Engel introduced additional parameters, especially regarding the titratable acidity.

I think the entire problem is more complicated in comparison to 1964, especially by the introduction of the SI units. The United States is lagging far behind the other countries, even though the other countries are still not quite uniform; for instance in England, they are going to use hemoglobin as grams per deciliter because of the confusio that would result with the use of millimoles. We have been lagging for the following reasons: number one, we're not in the metric system and, in reality, in order to get a metric system and SI units, we should be doing this with our school children rather than talking *ex cathedra* from this level to go down. We are really going to have our problem in the United States. In addition to this, we have a problem that we can accept it in t laboratory; I calculated that there are nine practicing clinical chemists in the audienc We can do it, but the fact is that the physician is not going to do it.

In addition to the problem of SI units, we have the problem of *in vivo* and *in vitro* diagnosis. The question of extra-cellular fluid regarding pH, especially, and blood gas Several years ago when there wasn't much distinction, they had a controversy. I think m and more today we're going over to the *in vivo* type of consideration and the clinicians going to have to learn to adjust.

Our third area is the question of temperature correction. To quote two good areas- Dr. Laver is not here now and we can say that his is a good area, Massachusetts General, the Mayo Clinic. They are routinely doing temperature correction for pH and blood gases all routine determinations as well as for the cardiac by-pass type of surgery. I think gave my stand yesterday on the plea that it should be done. The question of us today is what advantages would there be? We do not question as to what the normal would be at th patient's body temperature. We get the situation, such as Dr. Gambino has written about his monthly newsletter, of the patient coming in who has a PO_2 of over 150 breathing roo air; the physician says it's impossible, and when you check back, of course, that patien a low temperature. And finally, another area of controversy is the question of pH versus hydrogen ion concentration; and I won't make any comments about that.

Possible areas to be considered in greater depth by the subcommittees in the future perhaps would be P_{50}, or as our European friends call it T_{50}, oxygen content, and 2,3-DP But there has been some agreement. Number one, we agree whether pH or hydrogen ion woul be the area of determination for acidosis/alkalosis or acidemia/alkalemia. The PCO_2, or if you wish, the carbonic acid concentration or total CO_2 content by calculation would b reported for respiratory factors. We still have our century of the Tower of Babel in th we still lack agreement on which metabolic term and which metabolic test to be used. Whether the values should be reported per se, to use calculated values or those based on the determined ones, should be looked into to see what can be done. As a subgroup of th subcommittee A, is the question of the Evaluation of Nomograms and Algorithms. Here it was agreed, at least I think it was agreed, that there is a distinction between a diagra which would be a graphic representation for education and possible diagnostic help to th physician and the nomogram which is a means of calculating additional parameters for the

ratory personnel in order to report to the physician. Now it is not necessary to use agram or a nomogram in the better centers, but I think it does have some area of use he boondocks, if you would, where they don't have quite the expertise as this group today.

A subcommittee B would be entitled, with our subsections today, Blood Sampling, ling, and Storage. I think this is important enough to be a subsection of the proposed rt Panel and it may need a restatement of the state-of-the-art rather than any further stigation. But you heard the comments and the difficulties; as Dr. Ladenson showed, e are indefinite or insufficient data but there have to be more definitive areas of we're doing. I think here again, with my own personal bias, that for an evaluation O_2, you must have arterial blood, for the routine acid-base evaluation where you don't a PO_2, no matter how small that percentage, that venous blood would be perfectly sfactory. This has been substantiated time and time again. Dr. Gambino, of course, ne of the leaders in that area.

A subcommittee C would combine, I think, the Establishment of Reference Values, ity Control and Standards, and the Development of Reference Methods. And, as is bus from this afternoon, this was the area of greatest interest, contributed no doubt he fact that Mr. Cali gave his beautiful exposition of what the NBS does and so on. I k here again, the problems came up with tonometry. Should we use blood, an aqueous tion, or a glycerol solution as advocated by Dr. Maas? I think this requires a great of expert input, and we would have to have that subcommittee do that. The question ype of tonometer, and I apologize to Dan ahead of time, the simple Noonan tonometer, simple Noonan, but the simple Noonan-tonometer, versus the more complex ones. I think as agreed that the NBS should work in the areas of certifying gas mixtures for standardi- on for tonometry and for standardization of the instruments with a probable ultimate racy within one percent. It was also agreed, I believe, that the NBS should work in area of certifying buffers or buffer compounds for buffers of pH. I think the sugges- that was made is extremely important that these buffers be certified at 37 °C and we bracket the 7.40. It is crazy to have a pH in the low sixes and under 6.8, and so we really should be bracketing and my own preference would be somewhere about 7, 7.4 a 7.6 or a 7.7 in that area. It was agreed again to use an operational definition for As far as PCO_2 and PO_2 is concerned, there was some discussion for mass spectrometry, ner this is a definitive method or is this really a secondary method for accuracy. A metric Scholander-Riley technique would be the one to be utilized in the reference ratory. The problem came up of the question of normal levels for PCO_2 and PO_2 and, burse, that evolved itself with a give and take, but we won't mention the people ved. I believe, that an abnormal level, as it seemed to be the consensus, should sed primarily to check out instruments rather than for the beneficial knowledge to physician for diagnostic levels. Dr. Gambino reported on some data using General nostic's new aqueous acid-base control which contains PO_2 values, and this would nately replace the one that they have now which does not have a PO_2 and is a lyophilized rial, which I think has a great many problems attacked to it. If the liquid material s out well, as I think it will, it should be substantiated and supported. My own estion to Dr. Durst is that I think there have been enough people working with it, Gambino, Mr. Komjathy, and myself, that this should be included in the final mono- n, because you don't expect a company to publish somebody's in-house, out-of-the-house of work but with the monograph I think it will give some good data to show on that of support.

The next area of section D is the Instrument Specification and here, of course, we the best available and then beyond that. I think the consensus that everybody would e to is that we need better service manuals for maintenance and repair at all hours, ssible. But what we're really saying is we need a VIP instrument; VIP instrument ds for Very Idiot Proof. I think the manufacturers should be listening, as they were y, especially to that Section C with the Reference Methods and Standards, because even gh they are in the business to make money, we also have to have some of the accuracies g on. The future problem, as emphasized by Prof. Kreuzer, is the question of the *in* monitoring. Here, of course, with a PO_2 electrode that he described, micro and so can be extremely convenient. It was extremely interesting to see this new PCO_2 ctivity electrode because this is now opening some new parameters whereby we may make advances.

And finally, we have the Electrolytes. And this I find a little bit good and bad because it's sort of an afterthought. We had 2 days on the other groups and had only 2 hours for electrolytes. This is like the New Orleans type of talk of lagniappe, something extra for nothing. It came out very well because this is leading into my conclusion. And by taking it out of context, Dr. Brand gave a very nice talk and his comment that reporting the values as per liter of plasma water is extremely important. I think this has been suggested many times in the past. I was very pleased to hear Dr. Bates talk about sea water. Number one, because he is the other bald-headed, white-haired man in the audience, and number two, the sea water study that he is working with now takes me back to 1916 when the diagram (number 3 in my list) by McClendon also worked on sea water. So really we've gone full cycle now for another 60 years.

I wish to thank Dr. Durst for the honor of summing up the deliberations and, on behalf of all the participants, to congratulate him on being the honorary obstetrician for the birth and/or delivery of this conference. I hope that the monograph will be as successful as the one on Ion-Selective Electrodes.

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On July 7-8, 1975, a workshop was held at the National Bureau of Standards to discuss the status and needs of this very important area of clinical measurement. A major goal of this workshop was the initiation of cooperative efforts on an international level toward the standardization of pH and blood gas measurements and the various quantities and terms used in this field.

To this end, the first technical session was concerned with the acid-base status of blood and included the topics: Definitions of Quantities and Concepts; Recommendations of Nomenclature, Physiological Terminology and Symbols; Reference Values; and the Evaluation of Nomograms and Algorithms. The second session addressed itself to the more practical aspects of this subject and included the topics: Blood Sampling, Handling, and Storage; Instrument Specifications; Quality Control and Standards; and the Development of Reference Methods. Finally, a brief session on the newer topic of the electrometric measurement of blood electrolytes.

This volume contains all of the papers invited for presentation at the workshop by some of the leading clinical and medical authorities on this subject and also includes a transcription of the extensive discussion sessions.

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