CO2 and O2 equilibria in human blood and interstitial fluid

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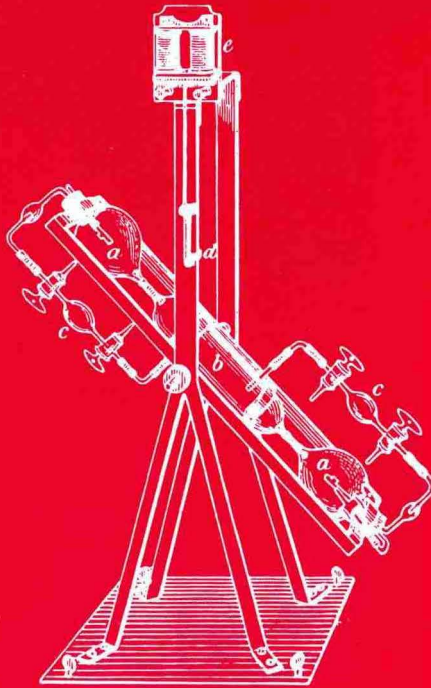
Jacob P. Zock

CO$_2$ AND O$_2$ EQUILIBRIA IN
HUMAN BLOOD AND INTERSTITIAL FLUID

Stellingen

1. Bij de beoordeling van het zuur-base evenwicht tijdens hypother-
   mie moet men uitgaan van bloedgasmetingen verricht bij de nor-
   male lichaamstemperatuur.

2. In aanwezigheid van CO_2 doen de N-terminale valines van de
   β-ketens van het humane hemoglobine mee bij het tot stand komen
   van het H^+ bohr effect.

3. Bij de berekening van snelle veranderingen van de pH in bloed is
   het onjuist in de Henderson-Hasselbalch vergelijking de "apparent
   over-all first dissociation constant" te gebruiken als niet zeker is
   dat koolzuuranhydrase in voldoende mate aanwezig is.

4. De sterke correlatie tussen de hoeveelheid hemoglobine in de rode
   bloedcellen en het oppervlak van deze cellen toont de grote
   functionaliteit van deze struktuur.

5. Een wezenlijke verrijking van de wetenschap door de computer is
   de mogelijkheid die deze biedt tot synthese van delen analytische
   kennis.

6. De optimale dagdosis voor vitamine C is voor veel mensen waar-
   schijnlijk een veelvoud van de aanbevolen dagdosis.

7. Op grond van het uitgebreidere huidcontact van urine bij vrou-
   wen is te verwachten dat overgevoeligheid voor sensibiliserende
   stoffen - aanwezig in voedings- en geneesmiddelen - die groten-
   deels onveranderd door de nieren worden uitgescheiden vaker bij
   vrouwen dan bij mannen zal voorkomen.

8. De emotionele en irrationele wijze waarop met het begrip discri-
   minatie veelal wordt omgegaan, maakt het gebruik van dit begrip
   tot middel van politieke manipulatie en staat oplossingen van
   interculturele problemen eerder in de weg dan dat zij ertoe bij-
   draagt.

9. Funktionarissen die hun beroepsmatige bestaansrecht ontlenen aan de eventuele noodzaak tot veranderen van een organisatie, dienen van deze organisatie geen deel uit te maken.

10. Het feit dat Nederlandse staatsburgers niet het recht hebben om door de Kroon uitgevaardigde wetten en besluiten door de onaf-hankelijke rechter aan de grondwet en internationale verdragen te laten toetsen maakt dat Nederland slechts in beperkte mate als een rechtsstaat kan worden beschouwd.

11. Dikdoeners in de sferen van wetenschap en industrie zijn onder andere te herkennen aan het veelvuldig gebruik van de term "technologie" als er "techniek" bedoeld wordt.

12. Promoveren na je veertigste is als verloven na je zeventigste.

Stellingen behorende bij het proefschrift "CO$_2$ and O$_2$ equilibria in human blood and interstitial fluid" van J.P. Zock.
Groningen, 1985.

CO$_2$ AND O$_2$ EQUILIBRIA IN HUMAN BLOOD AND INTERSTITIAL FLUID

PROEFSCHRIFT

ter verkrijging van het doctoraat in de Geneeskunde

aan de Rijksuniversiteit te Groningen

op gezag van de Rector Magnificus Dr. E. Bleumink

in het openbaar te verdedigen op woensdag 19 juni 1985

des namiddags te 4.00 uur

door

JACOB PHILIPPUS ZOCK

geboren te Dordrecht

To Jelly Mieke, Stijntje and Machteld
To my parents

CONTENTS

There are few things about scientific method that I hold more firmly than that accuracy beyond a reasonable factor of safety is a mistake in so far as one is concerned with means to an end.

L.J. Henderson
(as quoted by J. Parascandola)

CHAPTER I

INTRODUCTION

INTRODUCTION

A complex living organism like a human being can, in utmost reduction, be considered to consist of a very large number of the smallest units of life, the cells. These are surrounded by a fluid filling the interstitium - the space between them - and supported by the substance in it, which is produced and maintained by the cells. To keep alive, cells need energy and a suitable environment. Energy is derived from controlled combustion of nutrients. Both the nutrients and the oxygen needed for combustion are withdrawn from the interstitial fluid into which, in return, the cells dispose the degraded products of their metabolism. A suitable environment, therefore, has to contain sufficient nutrients and oxygen. Besides water, the main end product of aerobic energy liberation is carbon dioxide. In the interstitial fluid carbon dioxide is present in the form of the highly soluble CO_2 molecule and in the form of the HCO_3^- ion into which CO_2 is converted. In addition to nutrients and oxygen, the interstitial fluid contains electrolytes, of which the concentrations have to be kept within certain ranges. HCO_3^- is a buffer anion and thus contributes to the acid-base equilibrium. The composition of the interstitial fluid is continuously threatened by the uptake of nutrients by the cells and by the waste the cells return into it. These exchanges tend to change the composition of the interstitial fluid away from the optimum composition. For its constancy, the interstitial fluid is dependent on supply from and disposal into the circulating blood. Specialized organs like the lungs, the kidneys and the liver in their turn see to it that the composition of the onflowing arterial blood is adequate.

The transport of oxygen and carbon dioxide is the primary task of the blood. The transport of CO_2 is the result of close collaboration between the red cells and the blood plasma. The transport of oxygen, however, is almost completely done by the red cells. To this end, the red cells are packed with hemoglobin, a specialized protein. Oxygen, taken up in the lungs, is carried to the tissues where it is released. The release is furthered by the circumstances existing in actively metabolizing tissues to which the hemoglobin molecule is especially sensitive: a lower pH and a higher pCO_2 than in the arterial blood, both caused by the CO_2 produced by the cells. An additional factor is the rise in temperature. In the lungs, a reverse process is at work because the oxygen release by the blood in the presence of CO_2 has its complement in the CO_2 release in the presence of a higher than venous pO_2 in the lungs.

Although these processes have their origin in the special properties of the hemoglobin molecule, the comprehension of this interaction is complicated by the fact that the hemoglobin is not directly confronted with pH changes occurring in blood plasma. It is locked up inside the red cells in which the pH changes are modified by the equilibria across the red cell membrane. The

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change in these equilibria, furthermore, includes changes in the distribution of water and electrolytes, measureable as a change in the hematocrit. The interstitial fluid, for its part, is separated from the blood by the capillary wall. The changes around the tissue cells are thus transmitted to the blood in a modified form. This complicates the relation between changes in the neighbourhood of the cells and those in blood.

The interactions between the carbon dioxide and oxygen in blood and the interstitial fluid as outlined above, are the subject of this study. In our department a simple model of blood had been in use earlier. Starting with this model, several extensions and refinements have been introduced. These concern the introduction into the model of the osmotic and donnan equilibria across the red cell membrane and across the capillary wall, ATP and 2,3-DPG as buffer substances in the red cells, and a more detailed description of the functional properties of hemoglobin.

The transport of oxygen and carbon dioxide by the blood and the mutual interaction between them, and their connection with the distribution of water and electrolytes in blood have been the subjects of extensive research during the last two centuries. In the course of this period many fundamental concepts changed and new ones were introduced. In the last century a real revolution in thinking took place in the sense that vitalism was replaced by the notion that biophysical and biochemical processes form the basis of life. These new concepts were only gradually accepted.

In the course of his study on blood gas transport and acid-base equilibrium in the first part of our century, Lawrence J. Henderson came to realize that the complex interplay of the processes concerned forms a coherent physico-chemical system which can be described in terms of mathematical equations. This was a major advance, because experiments are necessarily restricted and can only concern a part of all the interactions since it is virtually impossible to measure all variables simultaneously. A mathematical description of the physico-chemical system would provide insight into its structure. In his famous book *Blood*, published in 1928, Henderson attempted such a description. The book is a compilation of his work on the subject of blood gas transport and blood acid-base balance (a term invented by Henderson). Besides presenting his own achievements, Henderson, in his book, summarized a great part of the insights gained in the research carried out in the preceding era.

Henderson begins chapter II thus: "The blood of vertebrates is a physico-chemical system of great complexity". Since then, this statement has proved to be far more true than Henderson may have imagined. Beside the gas transporting system, there are other systems in blood which illustrate its complexity, *e.g.* the hemostatic coagulation system. All the substances that are

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exchanged between cells are to some extent present in blood and many of them take part in a variety of interactions. The presence of these substances in blood plasma underlies the majority of the determinations in clinical chemistry and allows'the monitoring of many of the functions and processes in the body.

Henderson's statement has also remained true for the subject of his book, the gas transport by the blood and its connections with acid-base balance. Obviously, books treating only this subject cannot be given such a comprehensive title any longer and one may even wonder whether at the time of publication the title *Blood* was not already too wide for the subject treated. Nowadays, blood is a subject more suited to fill a whole library and not just one volume; and within that library a fair amount of room would be needed to accommodate the books on gas transport and acid-base balance. The number of studies concerning hemoglobin alone has grown immensely and today it is no longer possible for a single person to cover the whole field of gas transport and acid-base balance from its roots in the molecular properties of hemoglobin to the other, macroscopic, end of the spectrum concerning the gas transport to and from the tissues and the regulations thereof, together with all the other processes influencing acid-base balance.

The discovery of the role of oxygen in combustion by Lavoisier about two centuries ago can be considered the start of modern investigations into the subject of gas transport in the body. Lavoisier showed that the principle of combustion was not only valid in the anorganic world but in living beings as well. He proved the chemical nature of respiration in experiments on guinea pigs conducted in collaboration with Laplace. They measured the intake of oxygen and the output of CO_2 and heat, and showed that the continuous production of "animal heat" is a slow form of combustion.

For a long time it remained an open question at which site in the body heat was actually produced. In 1837, Gustav Magnus showed that this site could not be in the lungs because he found differences in O_2 and CO_2 content between arterial and venous blood. Around 1850 the common opinion was still that "animal heat" is produced in the red blood cells by combustion of nutrients. The heat was thought to be necessary to sustain the life of the tissues. A sufficient amount of red cells was therefore of prime importance for good health. The relation between the amount of red cells and nutrition had already been noted and good nutrition was considered to be of the utmost importance in illness. Anaemia itself was thought to be a cause of disease and not an eventual consequence of it. This may explain the emphasis put on anaemia even a long time after it had been found that the red cells were not the place where the heat was generated. The problem as to where the combustion occurs was tackled by a number of renowned scientists: Felix Hoppe-

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Seyler, Georg Liebrig, Heinrich Magnus and Eduard Pflüger. It was Pflüger who finally resolved the matter around 1870.

During the whole of the 19th century the developments in physiology were so closely connected with those in chemistry and physics that the demarcation between the disciplines was often not clear. Hemoglobin was already extensively being studied when Stokes, in 1864, discovered that the absorption spectrum of a hemoglobin solution changed when oxygen was withdrawn from it. At the time, it was already clear that hemoglobin played an important role in the oxygen transport by the blood. Stokes recognized that the blood's turning dark on passing from the arteries to the veins indicates that oxygen is used up. About ten years later Pflüger showed that this oxygen was given up by the blood and used up in the tissue cells.

In 1884 Hüfner determined the amount of oxygen bound by hemoglobin to be 1.34 mL per gram hemoglobin and in 1885 Zinoffski calculated on the basis of the measured iron content of horse hemoglobin that the molecular weight was I6670. From these values it was readily inferred that at high oxygen pressures one mole of hemoglobin binds one mole of oxygen. It was due to Christian Bohr that this insight was suppressed and had to be rediscovered by Barcroft and Peters some 25 years later. Bohr was convinced that several different forms of hemoglobin were present in one individual, each with a different composition and different properties. Bohr used this idea of multiple hemoglobins to explain the form of the oxygen-hemoglobin equilibrium curve.

Already in 1878 Paul Bert had published his finding that at 40 °C dog blood saturated with oxygen gave up about half of its oxygen when the partial pressure of oxygen fell to about 25 mmHg. Hüfner, on the other hand, came to a value of about 5 mmHg. His value was strongly biased by his assumption about the reaction equation. The matter was settled by Bohr and coworkers in 1904, who measured the complete relation between oxygen pressure and oxygen saturation and proved conclusively the influence of CO_2 on oxygen binding. Although Bohr is the first author, it is quite certain that the work was done by Krogh with the use of the tonometer designed by himself. It is a picture of this apparatus taken from Krogh's paper that is reproduced on the cover.

The measurements by Krogh confirmed the results of Paul Bert but raised the problem as to how the sigmoid curve should be explained. In 1910, Hill suggested that hemoglobin forms aggregates of variable numbers of molecules. Variability of the molecular weight and properties, of which Bohr was convinced, had already been rejected because of the osmotic pressure measured. In 1925 Adair reported new findings on the osmotic pressure exerted by hemoglobin in favour of a fixed molecular weight of four times the originally supposed value. This observation was supported by sedimentation measurements by Svedberg. On the basis of this new insight, Adair proposed that the

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binding of oxygen takes place in four steps, each with its own equilibrium constant. In 1955, Roughton and coworkers accurately determined the values of these four binding constants. These values indicated that a strong cooperativeness should exist in the binding of oxygen in the sense that once one oxygen molecule is bound, the affinity for oxygen of the other binding sites strongly increases. This phenomenon was explained by Monod, Changeux and Wyman in 1965 with the hypothesis that the quaternary structure of the hemoglobin molecule can be in one of two possible states. The states are indicated as the R and T state. The R state has a high oxygen affinity, the T state a low one. Perutz and coworkers, in 1971 and following years, showed with the aid of x-ray diffraction that the two states indeed existed.

The distinction between the two states of the hemoglobin molecule was in itself not new, what was new was only the interpretation given to it. Already in 1920 Henderson had made use of the assumption that the pK of some ionizable groups on the hemoglobin molecule would change under the influence of oxygen binding. The two states distinguished were oxyhemoglobin and deoxyhemoglobin (then called reduced hemoglobin). Less exactly formulated, but essentially the same, is the assumption by Christiansen, Douglas and Haldane (1914) that oxyhemoglobin is a stronger acid than hemoglobin. They proposed such a mechanism as a possible explanation of their findings that deoxygenated blood could accommodate more CO_2 than oxygenated blood at the same pCO_2. This assumption was not new either. Already in 1882, Nathan Zuntz had formulated this hypothesis to explain the disputed effect of O_2 on the amount of CO_2 expelled from blood.

With their results Christiansen *et al.* conclusively proved the influence of oxygenation on the CO_2 binding in the blood. In doing so they resolved a long-standing vexed question. In 1863 Holmgren, working with Ludwig in Vienna, observed that blood shaken with a gas mixture containing oxygen gives up much more CO_2 than when no oxygen is present. Afterwards Ludwig failed to confirm this finding. It was not without reason that Zuntz considered it a realistic possibility. In 1870 Zuntz had become an assistant to Pflüger in Bonn, where Wolffberg in 1871 confirmed the results of Holmgren. Zuntz published the above-mentioned explanation of this phenomenon in 1882. The effect of oxygen on CO_2 binding in blood was studied *in vivo* by Werigo in 1892. He separately ventilated the lungs of a dog and found that in the lung where hydrogen was used instead of oxygen, the pCO_2 was systematically lower. He discussed the physiological importance of this effect and postulated that in the tissues the reciprocal effect, *i.e.* CO_2 expelling O_2 from the blood, occurs. It was the latter effect which was eventually to be confirmed beyond doubt *in vitro* by Bohr *et al.* The experimental results of Werigo were eventually explained by the findings of Christiansen *et al.* The reciprocity that Werigo

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assumed was thus shown to exist.

In 1910 Barcroft and Orbeli found that lactic acid had the same effect on the oxygen equilibrium curve as CO_2. Therefore, the effect of CO_2 was explained by its properties as an acid. It was thus quite natural that the explanation of the expelling of CO_2 on oxygenation was sought in a change of the properties of the weak acid that hemoglobin is. Henderson had developed the theory of buffer action which enabled him to express this explanation in an exact way. It was the discovery by Christiansen *et al.* which made Henderson consider the blood as a physico-chemical system.

The reciprocity between oxygen binding and H^+ binding by hemoglobin was demonstrated more fundamentally by Wyman, who used chemical thermodynamics to formulate the relation in mathematical form in 1939. He extended his analysis further to include the influence of all substances that are bound by hemoglobin on the oxygen affinity of hemoglobin. This theory was published in 1964 and is used in the present study.

Wyman's theory could thus also be used to explain other interactions than that with protons. About 1930 it had been shown that CO_2 itself binds to hemoglobin. This binding appeared to depend on oxygenation too. It follows from Wyman's relations that CO_2 exerts its influence on the oxygen affinity of hemoglobin not only by means of proton release but also in a direct way. This has been confirmed experimentally. Wyman's theory also applies to another ligand of hemoglobin, 2,3-diphosphoglycerate. Its binding to hemoglobin was discovered only fairly recently, *i.e.* in 1967.

CO_2 in the blood is distributed over the red cells and plasma. To be useful for the transport of CO_2 the distribution has to occur very rapidly. Several discoveries and new concepts in physical chemistry were needed before this process could be completely understood. Until 1887, when Arrhenius published his theory on the dissociation of electrolytes, transfer of bicarbonate and chloride was thought to be possible only in the combination as a salt as proposed by Zuntz, or an acid as proposed by Gürber. The latter observed that the red cell membrane was impermeable to sodium and potassium, but that exchange of chloride and bicarbonate did take place. In 1897 Koeppe explained this by assuming that $CO_2^=$ is exchanged against Cl^-. The observation that CO_2, in combination with deoxygenation, makes red cells swell and take up chloride is known as the Hamburger shift, after Hamburger, who published his findings in 1891. The same observation had already been made in 1878 by Herman Nasse, but this remained unnoticed. Neither he nor Hamburger could properly explain the phenomenon. The fact that this shift has nevertheless been called "Hamburger shift" is probably due to the fact that Hamburger became well known by the book he wrote in 1902 on osmotic pressure and the importance of ionic dissociation. Hamburger himself refers to Koeppe and

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Gürber for the explanation of his observations and admits that in 1891 he, too, supposed that the exchange took place in the form of salts.

For the study of the distribution of CO_2 between cells and plasma the concept of what is now called the donnan equilibrium was important. Donnan published it in 1911, shortly after Sørensen's concept of pH (1907) and Henderson's theory of buffer action (1908). This made it possible to study the "heterogeneous equilibria", as Henderson called them, between red cell content and plasma. A major improvement was the conversion of Henderson's buffer equation into a logarithmic form by Hasselbalch in 1917. This equation, which underlies the whole description of the acid-base balance, is therefore called the Henderson-Hasselbalch equation. Around 1920, this branch of physiology became more and more quantitative and many scientists devoted long years of work to measure the relations between the quantities relevant to the subject.

The subject of the present study is thus not new in physiology, and it may even be considered to be rather old-fashioned. The goal is the same as that aimed at by Henderson, but with the application of modern means and supplemented with insights gained since then. As already mentioned, Henderson tried to cope with the numerous relations governing the physico-chemical equilibria involved in the gas transport by the blood. He did this by using various nomograms representing these relations. The set of nomograms designed by Henderson can be considered to be a model of the physico-chemical properties of blood as far as gas transport and acid-base balance are concerned. Looked at in this way, the model incorporates the state of knowledge then prevailing about these subjects.

In Henderson's time the word "model" had not yet been coined to cover the notion of a description of a complex system. A model can be regarded as a representation of a certain part of reality with respect to some specific properties of that part of the reality. This representation can take different forms. An animal upon which a disease has been inflicted and which is used for studying the course of the disease as it might occur in a human being, is a model of the infected human being. A lump of tissue or a line of cultivated human or animal cells may sometimes also be used to simulate such a course of events and is then used as a model. In medical education, models of parts of the human body are used to simulate the relevant anatomical relations in order to train the skills needed for certain investigations or interventions. In other disciplines planes, ships, bridges are built to scale in order to study various properties of the real objects such as the influence of their shapes, their behaviour when loaded, etc. River deltas are built to scale, as are chemical plants. All of them are models used to represent and study certain parts of reality.

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The models mentioned are real objects themselves, differing from their originals mainly in scale. In quite another way, mathematical formulae can be used to represent the parts of reality we are interested in. The behaviour of a gas, for instance, can to a certain degree be described by the gas law: $pV = nRT$. Just as is the case with equivalent models representing objects to scale, this representation is only accurate within certain limits. Up to these limits only the restricted number of quantities occurring in the equation are needed to represent the part of reality under study. When the values of these quantities are known, the state of this part of reality fixed.

In order to obtain a better description of reality it might be necessary to use more, or more extended, equations. This may entail an increase in the number of variables, but not necessarily so. The gas law, for instance, can be improved in the way given by Van der Waals. The Van der Waals equation differs from the gas law in the number of constants used, not in the number of variables. Though it may seem somewhat exaggerated to call an equation like the gas equation a "model" where it is possible to calculate with simple means every possible combination of values that the quantities may take, it may, nevertheless, be considered an example of a mathematical model. This notion of a mathematical model is more evident in the case where the equations that determine the state of a system increase either in number or in complexity. Obtaining an analytic solution may then become prohibitively laborious, thus necessitating other ways of finding solutions.

Before the surge of the digital computer, several ways to accomplish this were in use. Often another real physical system was built that obeyed equivalent equations. The advantage of the substitute system over the original one was that in it the quantities of interest could be measured more easily or that the scale was more suitable as regards size or costs. One of the most versatile and generally applicable systems of this kind of equivalent system is the analogue computer, in which variables are represented by the electrical quantities potential and current. Although still in use for special purposes, the analogue computer has lost most of its ground to the digital computer, which can be completely manipulated by the input of strings of characters without the need of rewiring and connecting new apparatus on each different application.

Although in many cases not of practical importance, it must be realized that quantities which in reality can take an uncountable infinite number of values are represented in the digital computer by quantities that can take only a countable infinite number of values owing to the way numbers are represented. Consequently, in the end rounding off errors can build up to disturbing levels and lead to unreliable results.

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The digital computer makes it possible that once the proper set of equations and constants are at hand, the mathematical model can be realized by implementing them in a computer program. Running this program leads to an imitation of reality expressed in numbers. This is called a computer simulation. The use of a computer simulation to study the behaviour of a system has a number of advantages. When the program is ready in the computer, experiments can be performed many times for different values of the parameters with only a limited extra effort. If the program is well designed, relatively few special skills are required to run an experiment. Unlike in a real experiment extreme conditions can be imposed as easily as normal ones. A multitude of quantities can be observed simultaneously.

For the solution of numerical problems presenting themselves in a model one can rely on standard programs from numerical libraries. The advantages of a mathematical model are not restricted to its practical use alone. As far as the subject itself is concerned, a mathematical model forces one to define all parts neatly. Lacunas in the knowledge of the system which is to be represented in the model become directly apparent. Interactions between parts can be changed and if redundant data are incorporated internal inconsistencies appear. Models are therefore well-suited to support research by making possible the synthesis of different parts into a whole. After its completion a model can be transformed into an educational tool in which a vast amount of knowledge is compiled.

The present study is part of the research going on in the Department of Medical Physiology of the University of Groningen. Preceding studies by Brunsting and Rispens concerned the way quantities related to CO_2 in blood as well as in respiratory gas can be measured. Furthermore, his thesis also deals with the interpretation of those quantities in blood when measured in the clinical laboratory. In his study a number of computations were needed to support the analysis. Most of the programs for these calculations were written by the present author, who, after a delay of some years, has continued this line of research, which has resulted in the present study.

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CHAPTER II

CARBON DIOXIDE LOADING AND THE ACID-BASE
EQUILIBRIUM STATES OF HUMAN BLOOD

Carbon dioxide loading and the acid-base equilibrium states of human blood*.   I

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ABSTRACT

To calculate acid-base equilibrium states of human blood in connection with blood gas transport, relations and quantities concerning related physico-chemical properties were collected from the literature. These included the Henderson-Hasselbalch equation, and the amount of charge of plasma protein and of hemoglobin varying with pH, the charge of hemoglobin also being dependent on oxygen saturation and carbon dioxide tension. Also included were the Gibbs-Donnan distribution of hydrogen ion activity and of chloride and bicarbonate ions, and osmotic pressure equilibrium conditions. Moreover, the temperature dependence of all relations was taken into consideration. The expression used for the osmotic pressure of hemoglobin was based on osmotic behaviour of albumin as given by Landis and Pappenheimer rather than on the values of Adair. The equilibrium model formed by these equations and quantities describes two homogeneous solutions, one being plasma and the other the erythrocyte content, the two being separated by a membrane which was assumed to be impermeable to sodium, potassium, proteins and organic phosphates. This model was implemented as a computer program. Various characteristic phenomena concerning blood gas transport and acid-base equilibrium could be calculated with this model, *e.g.* the plasma bicarbonate-pH relation, the changes of hematocrit with pH, and the carbon dioxide saturation curve of blood — all of them dependent on oxygen saturation and temperature. The results agreed well with experimentally determined relations and values as given in the literature. The results suggest that the hitherto accepted osmotic pressure relation of hemoglobin may not be the true one.

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Gas transport by the blood is closely connected with acid-base balance, because carbon dioxide constitutes the bulk of the acid carried by the blood and because of the interrelationship of oxygen transport and blood pH. Inside the red cells CO_2 is rapidly converted into H_2CO_3 by carbonic anhydrase. Within the range of pH encountered in the blood virtually all H_2CO_3 dissociates into HCO_3^- and H^+. Inside the erythrocytes H^+ is buffered by hemoglobin and to a lesser extent by organic phosphates; a small part of it is carried across the cell membrane (41) and is buffered by plasma proteins. The excess HCO_3^- inside the red cells moves across the membrane into the plasma. CO_2 is also directly bound to amino groups of hemoglobin transforming them into carbamino groups. Because the formation of carbamate turns a basic group into an acidic group, hemoglobin becomes a stronger acid with the binding of CO_2. The equilibrium constants of the reactions involved are dependent on the oxygenation of hemoglobin. Besides, hemoglobin becomes a stronger acid on oxygenation. Therefore, at a certain pH the net charge of hemoglobin is dependent on its oxygen saturation and the amount of bound CO_2. This is reflected as a change in the titration curve (66, 68). The binding of CO_2 by hemoglobin is dependent in a yet not quite elucidated way on the concentration of organic phosphate compounds in the erythrocytes (10, 44). Among these phosphates 2,3-diphosphoglycerate (2,3-DPG, in subscripts DPG) appears to be most important. Moreover, all equilibria are influenced by temperature. Temperature is an important variable in man because homeothermia does not imply that the body is isothermal (55, 57). All factors taken together, the net negative charge of hemoglobin depends on temperature, oxygen saturation, pH, CO_2 tension and 2,3-DPG concentration in the erythrocytes.

Hemoglobin cannot pass the cell membrane and therefore its charge is fixed inside the red cells, and as a consequence there is a Donnan distribution of the ions that can freely pass the membrane. The required electroneutrality within the two compartments, plasma and erythrocytes, gives rise to a net displacement of the diffusible anions – mainly Cl^- and HCO_3^- – across the cell membrane when changes of pH, oxygen saturation and CO_2 tension occur. The kations – mainly Na^+ in plasma and K^+ in the erythrocytes – are actively confined to their compartments. The net displacement of diffusible anions tends to change the osmotic pressure inside and outside the cells. A net displacement of water accompanying the anion shift keeps the total osmotic pressure inside and outside the cells equal (18, 42, 69).

The above-mentioned processes influence each other in many ways, which makes it difficult to get an overall picture of the events occurring during loading and unloading of blood with carbon dioxide and oxygen, even when these events are simplified to a succession of equilibrium states. Until recently such calculations were too elaborate to make them practically possible. Calculations of this type have been made earlier (21, 36, 78, 80), but a detailed quantitative treatment of the subject became possible only with the use of computers. Studies of this kind have been made by Lloyd and Michel (48), Roos and

Thomas (64), Dell and Winters (17), Rispens (59), Thomas (75), Siggaard-Andersen (72) and Brodda (13) and recently by Rodeau and Malan (63). The model presented in this paper is a continuation and extension of the work of Rispens.

MODEL

In the model the equilibrium conditions of the above-mentioned processes are described in a quantitative way by means of equations with proper values of the parameters. This makes the implementation in a computer program possible.

In the model, blood is a suspension of erythrocytes in plasma. Plasma is thought to be a homogeneous solution of $NaCl$, $NaHCO_3$ and proteins in water. The red cells consist of a selectively permeable membrane containing the red cell content. Red cell content is assumed to consist of a homogeneous solution of KCl, $KHCO_3$, adenosine-triphosphate (ATP), 2,3-DPG and hemoglobin in water (32, 82). Na^+ represents all kations of plasma, K^+ stands for all kations actually present in the erythrocytes. Na^+ and K^+ are assumed to be completely confined to their compartments. The red cell membrane is taken to be impermeable to plasma proteins, hemoglobin, ATP and 2,3-DPG, and freely permeable to CO_2, H_2O, Cl^- and HCO_3^- (33). This means that at equilibrium the erythrocytes are in osmotic equilibrium with plasma (38, 56). In the model the red cell membrane has no other properties (Fig. 1).

The oxygen saturation of hemoglobin is given a certain value. The amount of dissolved oxygen is neglected. In this way the complications concerning the
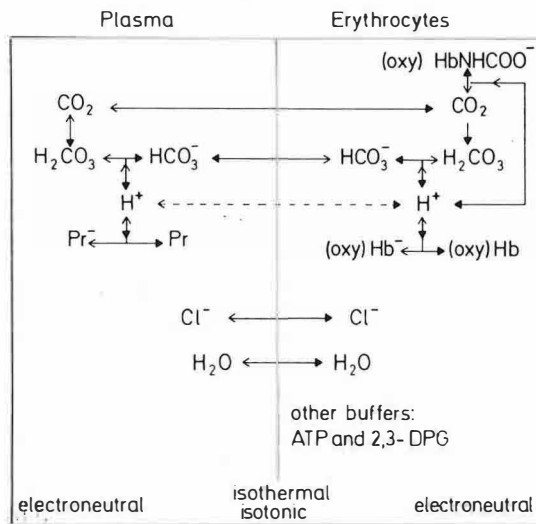

Fig. 1. Schematic representation of the equilibria considered in the model. Closed arrows indicate the direction in which the equilibria shift when CO_2 is added; open arrows indicate the shift when CO_2 is withdrawn.

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relation between oxygen pressure and oxygen saturation of hemoglobin and the influence of pH, pCO_2 and 2,3-DPG thereupon are circumvented. In the model it is assumed that the temperature is the same throughout the blood.

The electroneutrality of both solutions – plasma and erythrocyte content – requires that

$$(1) \qquad n_{p,Na^+} = n_{p,Cl^-} + n_{p,HCO_3^-} + z_{Pr} \cdot w_{p,Pr}$$

$$(2) \qquad n_{c,K^+} = n_{c,Cl^-} + n_{c,HCO_3^-} + z_{ATP} \cdot n_{c,ATP} + z_{DPG} \cdot n_{c,DPG} + \\ + z_{Hb} \cdot n_{c,Hb} + z_{HbO_2} \cdot n_{c,HbO_2}$$

where n denotes an amount of substance in mole, w a mass in gram. The index p means plasma, c stands for red cell; z is the amount of charge per mole, except for plasma protein where it is the amount of negative charge per gram.

Cl^- and HCO_3^- are assumed to be distributed between plasma water and red cell water according to the Gibbs-Donnan equilibrium conditions:

$$(3) \qquad r_{c,p} = \frac{c_{cw,Cl^-}}{c_{pw,Cl^-}} = \frac{c_{cw,HCO_3^-}}{c_{pw,HCO_3^-}}$$

$r_{c,p}$ is the Donnan ratio and c_{cw}, c_{pw} denote the substance concentration in cell water and plasma water, respectively. The partition of proton activity a_{H^+} is also assumed to be according to the Gibbs-Donnan condition, so that

$$(4) \qquad \log(r_{c,p}) = pH_c - pH_p$$

The osmotic equilibrium condition is

$$(5) \qquad \begin{cases} \phi_i \cdot RT \cdot \left(\dfrac{n_{p,Na^+} + n_{p,Cl^-} + n_{p,HCO_3^-}}{w_{p,H_2O}} \right) + \pi_{p,Pr} = \\[2ex] = \phi_i \cdot RT \cdot \left(\dfrac{n_{c,K^+} + n_{c,Cl^-} - n_{c,HCO_3^-} + n_{c,ATP} + n_{c,DPG}}{w_{c,H_2O}} \right) + \pi_{c,Hb_4} \end{cases}$$

where ϕ_i is the osmotic activity coefficient of the ions, $\pi_{p,Pr}$ the plasma protein osmotic pressure, π_{c,Hb_4} the hemoglobin osmotic pressure and w_{p,H_2O}, w_{c,H_2O} the mass of plasma water and of cell water*.

The relation between plasma pH, plasma pCO_2 and plasma HCO_3^- concentration is given by the Henderson-Hasselbalch equation:

$$(6) \qquad pH_p = pK_1' + \log \frac{c_{p,HCO_3^-}}{S' \cdot pCO_2}$$

where c_{p,HCO_3^-} is the plasma bicarbonate concentration in $mmol \cdot l^{-1}$ and S' the solubility of carbon dioxide in plasma in $mmol \cdot l^{-1} \cdot kPa^{-1}$. The values of pK_1' and S' are dependent on temperature. According to Rispens et al. (60) this dependence is fairly approximated by

$$(7) \qquad pK_1' = -4.7416 + 1840.11/T + 0.015906T - \log\left(1 + \frac{0.020682}{10^{(7-pH_p(T))}}\right)$$

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* Throughout this paper Hb_4 means hemoglobin tetramer and Hb hemoglobin monomer.

where T is temperature in K. A similar equation has been given by Reeves (57).

Only in the lower temperature range the two equations give slightly different values of pK'. The solubility of carbon dioxide in relation to temperature is given by

(8) $\qquad S' = 7.50\,(-3.246 + 536.73/T + 0.004985\,T)$

Rispens (59) arrives at this expression using values given by Austin *et al.* (6).

Given suitable expressions for the titration curves of plasma proteins, hemoglobin, ATP and 2,3-DPG in relation to the variables on which they depend and given the osmotic activity coefficients and the relation between protein concentration and water content of a solution, the above equations together with proper starting values are sufficient for calculating the interdependence of oxygen saturation (S_{O_2}), carbon dioxide tension (pCO_2), plasma pH (pH_p), blood CO_2 content (c_{b,CO_2}) and temperature (T_b).

The negative charge per gram plasma protein z_{P_r} depends on plasma pH, as given by the titration curve of the protein. This curve is usually assumed to be a straight line in the pH range 6.8–8.0. The slope of this line is 0.107 mole of charge per gram per pH unit (47). The iso-electric point of plasma protein used in the model is 5.1 at 37°C (77). The titration curve at 37°C is thus represented by

(9) $\qquad z_{p,Pr} = 0.107\,(pH_p - 5.10)$

The slope of this curve is commonly assumed to be independent of temperature. We use a change in iso-electric pH of -0.014 per °C, according to Rispens (59).

The titration curve of hemoglobin depends on oxygen saturation, carbon dioxide tension, 2,3-DPG concentration and temperature (10, 44). The influence of oxygenation and carbon dioxide binding on the titration curve of hemoglobin are treated as independent effects, superimposed upon the titration curve of deoxygenated hemoglobin in the absence of CO_2. The latter is fairly approximated by a straight line. Various values have been reported for the iso-electric point of deoxygenated hemoglobin and the slope of this line (27, 28, 35, 44, 66, 72). In the model we use an iso-electric pH of 6.81 and a slope of 2.7 mole of charge per mole of deoxygenated hemoglobin per unit pH*. Thus in the absence of CO_2 the titration curve of deoxygenated hemoglobin is described by

(10) $\qquad z_{Hb} = 2.7\,(pH_c - 6.81)$

The temperature dependence of this slope is -0.009 pHu^{-1}·K^{-1} (5). The iso-electric point of deoxygenated hemoglobin changes by -0.0122 pHu·K^{-1} (5).

The titration curve of oxygenated hemoglobin differs slightly from that of deoxygenated hemoglobin. This difference can be attributed to changes in equilibrium constants of alkaline and acidic Bohr groups on the hemoglobin molecule with oxygenation (5, 64, 66, 84). Therefore, each type of group is

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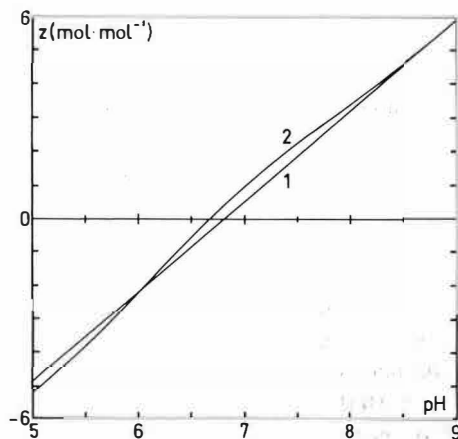* One unit of pH will be abbreviated as pHu.

Fig. 2. Net negative charge per mole Hb (z) in the absence of CO_2 plotted against pH; curve 1: deoxygenated hemoglobin, curve 2: oxygenated hemoglobin.

characterized by two equilibrium constants, one for deoxygenated hemoglobin and one for oxygenated hemoglobin. The difference in the charge on one mole deoxygenated hemoglobin and one mole oxygenated hemoglobin is calculated with the use of these four constants. The charge on one mole oxygenated hemoglobin in the absence of CO_2 is then obtained by the addition of this difference to the amount of charge on one mole deoxygenated hemoglobin (Fig. 2). The values for the constants are for the acidic group $pK_{Hb} = 5.4$, $pK_{HbO_2} = 5.9$, and for the alkaline group $pK_{Hb} = 7.5$, $pK_{HbO_2} = 6.45$ (5, 44). The temperature dependence of the pK' of the alkaline Bohr groups is -0.0167 K^{-1} for oxygenated hemoglobin and -0.0265 K^{-1} for deoxygenated hemoglobin (44). The temperature dependence of the acidic Bohr groups is 0.004 K^{-1} (5).

The binding of CO_2 by hemoglobin influences the titration curve of hemoglobin, because an alkaline amino group is turned into an acidic carbamino group. From the equations describing these reaction, it follows that (66)

$$(11) \qquad Z = \frac{K_c' \cdot K_z' \cdot c_{c,CO_2}}{K_c' \cdot K_z' \cdot c_{c,CO_2} + K_z' \cdot a_{H^+} + (a_{H^+})^2}$$

where Z is the CO_2 saturation of hemoglobin (Fig. 3, Fig. 4), c_{c,CO_2} is the concentration of dissolved CO_2 ($\text{mol} \cdot \text{l}^{-1}$) and a_{H^+} the proton activity ($\text{mol} \cdot \text{l}^{-1}$). The equilibrium constants K_c and K_z are different for oxygenated and deoxygenated hemoglobin. We resolved the problem of the partially oxygenated hemoglobin molecules by considering them as a mixture of fully saturated and completely deoxygenated hemoglobin molecules and treating both components as being mutually independent. The reactions are presently the subject of much research and at the moment there are no generally accepted values for the equilibrium constants. Perella et al. (53) do not give values for oxygenated hemoglobin. The values stated by Bauer and Schröder (11) result in

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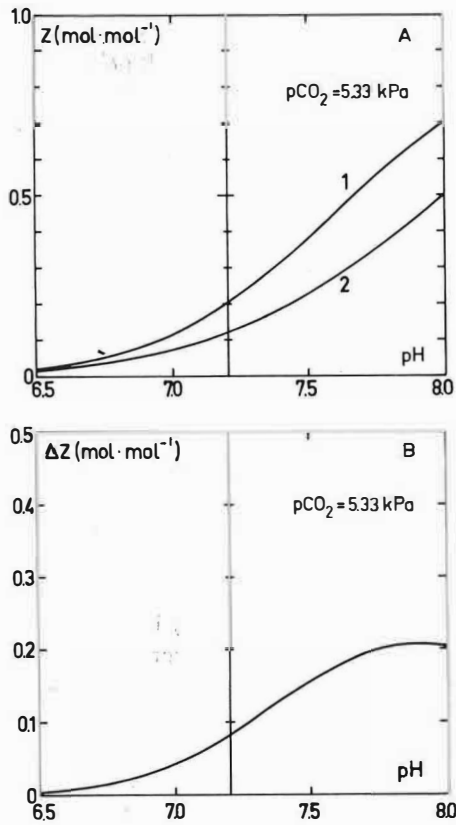
Fig. 3. A. Amount of carbamate per mole Hb (Z) plotted against pH; curve 1: deoxygenated hemoglobin, curve 2: oxygenated hemoglobin. B. Difference between the amounts of carbamate of deoxygenated and oxygenated hemoglobin per mole ($\triangle Z$) plotted against pH.

Fig. 4. Difference in net negative charge between one mole of oxygenated and one mole of deoxygenated hemoglobin ($\triangle z$) plottted against pH; curve 1: $p\mathrm{CO_2} = 0$, curve 2: $p\mathrm{CO_2} = 5.33$, curve 3: $p\mathrm{CO_2} = 10.7$ kPa (0, 40, 80 mmHg).

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very low carbamino-hemoglobin amounts in oxygenated blood. We used the values obtained by Klocke (45) for human blood: $pK_{c,Hb} = 4.6$, $pK_{c,HbO_2} = 5.0$, $pK_{z,Hb} = 7.0$, $pK_{z,HbO_2} = 6.5$. The temperature dependence of t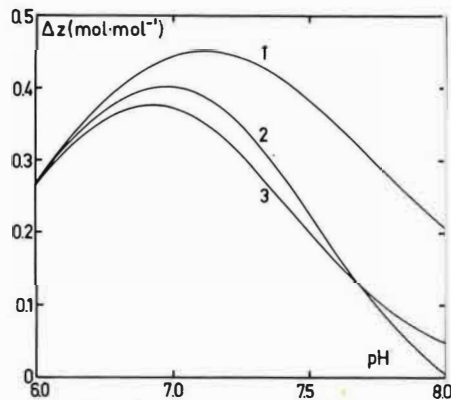hese constants is not mentioned. Therefore, the temperature coefficient of $pK_{c,Hb}$ and pK_{c,HbO_2} was supposed to be equal to the one of bovine hemoglobin, *i.e.* 0.019 K^{-1} (67). Because the corresponding values of pK_z are not mentioned, the temperature dependence was provisionally taken to be equal to that of the pK of the alkaline Bohr group *i.e.* -0.022 K^{-1} (5).

The matter is further complicated by the influence exerted by organic phosphate compounds in the erythrocytes upon the formation of carbamino-hemoglobin. At the moment there is no generally accepted opinion regarding the reaction equations and equilibrium constants describing the influence of 2,3-DPG on the titration curve of hemoglobin. In a tentative formulation by Kilmartin and Rossi-Bernardi (44) no values of the reaction constants are presented. Hence, the influence of 2,3-DPG on carbamino formation was implicitly included in the model.

The concentration of dissolved CO_2 in the erythrocytes was calculated using a solubility of 195 $nmol \cdot l^{-1} \cdot Pa^{-1}$ (0.0260 $mmol \cdot l^{-1} \cdot mmHg^{-1}$) at $37°C$ (79). The temperature variation of this value was supposed to be in proportion with the temperature variation of the CO_2 solubility in plasma.

The charges on ATP and 2,3-DPG are represented by z_{ATP} and z_{DPG} in equation 2. The values for the corresponding pK's and their temperature dependence were taken from Reeves (58). In the physiological range of pH both ATP and 2,3-DPG have three groups with fixed negative charge. ATP has one dissociable acidic group with $pK' = 7.04$ and $dpK'/dT = -0.0124$, and 2,3-DPG has two dissociable acidic groups both with $pK' = 6.73$ and $dpK'/dT = 0.0026$.

The red cell is in osmotic equilibrium with plasma (38, 56). Osmotic pressure is the sum of the colloid osmotic pressure and the osmotic pressure caused by the cristalloids in the solution. The latter is

(12) $\qquad \pi_c = R \cdot T \cdot \Sigma(\phi_i \cdot m_i)$

where π_c is the osmotic pressure caused by the cristalloids, R the gas constant, T temperature, ϕ_i the molal osmotic coefficient and m_i the molality of substance i. For a 0.120 mol/l NaCl solution in water, $\phi = 0.92$ and for a 0.08 mol/l KCl solution in water, $\phi = 0.913$ at 310 K (16). As the influence of HCO_3^- upon these values is uncertain, $\phi_i = 0.92$ is provisionally used in equation 12 for all ions in solution.

The osmotic pressure caused by proteins in solution is not linearly related to protein concentration (19, 46, 49, 70). The osmotic coefficient is larger than 1 and depends on concentration. This can partly be explained by the fact that the protein molecules are much bigger than water molecules and thus prevent a fraction of the volume from being occupied by the solvent (39, 71).

Landis and Pappenheimer (46) give empirical expressions for the osmotic

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pressure of albumin solutions and plasma over the range of 0 to 25 g·dl⁻¹, based on data from several sources. These expressions are

(13) $\pi_{Alb} = 2.8\ c_{Alb} + 0.18\ c^2_{Alb} + 0.012\ c^3_{Alb}$

(14) $\pi_{plasma} = 2.1\ c_{p,Pr} + 0.16\ c^2_{p,Pr} + 0.009\ c^3_{p,Pr}$

where π is pressure in mmHg and c is protein concentration in g·dl⁻¹. The dependence of the osmotic coefficient on concentration is implicit. Changing over to concentration in g·l⁻¹ and pressure in kPa, and writing the osmotic coefficient between brackets, gives for albumin solutions:

(15) $\pi_{Alb} = 0.037\ c_{Alb}\ (1 + 6.4\ 10^{-3}\ c_{Alb} + 42.9\ 10^{-6}\ c^2_{Alb})$

At infinite dilution the osmotic coefficient approaches 1, and $\pi_{Alb} \rightarrow R \cdot T \cdot c_{Alb}/M_{Alb}$, where M_{Alb} is the relative molecular mass of albumin, and $T = 310$ K. The molecular mass of hemoglobin being 64 458, the relation between the osmotic pressure caused by hemoglobin at infinite dilution and the concentration is

(16) $\pi_{Hb_4} = R \cdot T \cdot c_{Hb_4}/M_{Hb_4} = 0.040\ c_{Hb_4}$ (at 310 K)

At finite dilution the osmotic coefficient of hemoglobin has to be taken into account. Because of the small difference between the molecular masses of albumin (69 100) and hemoglobin (64 458) and because both molecules are spherical, we used the expression for the osmotic coefficient of albumin. The osmotic pressure of a hemoglobin solution is thus calculated according to

(17) $\pi_{Hb_4} = 0.040\ c_{Hb_4} + 2.57\ 10^{-4}\ c^2_{Hb_4} + 1.71\ 10^{-6}\ c^3_{Hb_4}$

The colloid osmotic pressure of plasma is calculated with an adapted version of equation 14 (Fig. 5). The total osmotic pressure of a solution of electrolytes and
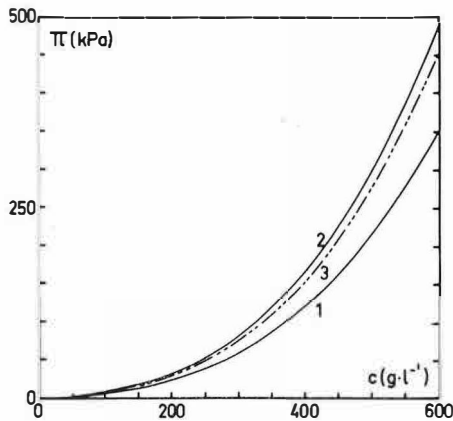

Fig. 5. Relations between colloid osmotic pressure (π) and concentration (c) as used in the model; curve 1: plasma protein, curve 2: hemoglobin, curve 3: albumin (46).

proteins in water is obtained as the sum of both. Osmotic pressure caused by crystalloids depends on molality, colloid osmotic pressure is according to the above-mentioned expressions calculated with concentration given as mass per volume of solution. It is therefore necessary to know the amount of water contained in a protein solution. The water fraction in normal erythrocytes has been determined by several investigators. Some older values are 0.73 mentioned by Van Slyke *et al.* (79) and 0.72 which can be derived from values given by Dill *et al.* (21). Savits *et al.* (70) find a water fraction of 0.717 l water per liter solution. One of the seven determinations by Hald *et al.* (33) has obviously been done on blood of an anaemic person. Omitting this value and taking the mean of the remaining ones also gives 0.717. The water content found by Kessler *et al.* (43) as well as by Eisenman *et al.* (22) is 713 $g \cdot l^{-1}$. The density of water at 37°C being 0.993, the water fraction thus determined is 0.718.

The water content of plasma containing 70 $g \cdot l^{-1}$ protein is 934 $g \cdot l^{-1}$ (22), corresponding to a water fraction of 0.9406. At 37°C the amount of water in one liter of a 160 $mmol \cdot l^{-1}$ NaCl solution differs insignificantly from the amount of water in one liter of pure water. In a physiological protein solution the non-water volume is thus completely occupied by the protein. The protein volume fraction in the erythrocyte is, consequently, 0.283 and in plasma containing 70 $g \cdot l^{-1}$ protein 0.0594. In the erythrocytes this includes non-hemoglobin protein and in plasma the plasma lipids. Their volumes are supposed to be included in the hemoglobin volume and plasma protein volume, respectively. The mean hemoglobin concentration in the erythrocytes of healthy men is about 333 $g \cdot l^{-1}$ (4, 31, 54, 81). The volume occupied by 1 g of hemoglobin is thus $283/333 = 0.850$ ml. The volume occupied by 1 g of plasma protein is $59.4/70 = 0.849$ ml.

These results mean that in the model the volume occupied by 1 g of protein in plasma as well as by 1 g of hemoglobin in the erythrocyte can be taken to be equal and independent of protein concentration. The relation between water fraction (F_w) and mass concentration of protein is thus given by

(18) $F_w = 1 - 0.85 \ 10^{-3} \ c_{Pr}$

where c_{Pr} is the mass concentration protein in $g \cdot l^{-1}$ of solution. The water content (c_w) of such a protein solution is

(19) $c_w = 0.993 \ F_w$

kg water per liter of solution (at 310 K).

The above-mentioned equations link the variables so that only a restricted number of them can be freely chosen or varied independently. To start the computations, a boundary condition must be given; to assess the consequences of variations, a reference or standard state is needed. We took standard oxygenated blood, *i.e.* blood with $S_{O_2} = 1$, $pCO_2 = 5.33$ kPa (40 mmHg), $pH_p = 7.410$ (71) and $T = 310$ K as the boundary condition and as the reference state. Once these variables were fixed, an independent value could be given to only six other variables. In the model at the standard state we took the

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following values: $pH_c = 7.202$ (81), $c_{p,Na} = 0.153$ mol·l^{-1} (Na represents all kations) (Table 2), $c_{p,Pr} = 70$ g·l^{-1}, $c_{b,Hb} = 150$ g·l^{-1}, $c_{c,Hb} = 333$ g·l^{-1}, $c_{cw,ATP} = 1.5$ mmol·l^{-1} (9). Insertion of these values into the equations fixes the standard state. Changes following variation of one or more variables were computed and compared with the standard state.

CALCULATIONS

With the aid of the presented equations and the chosen values the amounts of blood chloride, cell potassium and cell 2,3-DPG were calculated for the standard state. Once the standard state was known, one or more quantities were varied and the resulting change of the equilibria calculated. *E.g.* changing the pCO_2 resulted in a change in plasma pH and in plasma bicarbonate concentration. A plot of these two quantities is the blood bufferline in the plasma bicarbonate-pH (Van Slyke-Davenport) diagram. The results of the calculations are presented in the next section. The set of equations could not always be solved directly. In that case the solution was found by consecutive approximations. The maximal permitted error in the solutions corresponded with 0.0002 pHu. The calculations were interrupted when the conditions set by the equations were satisfied to the chosen precision.

RESULTS

The calculated composition of human blood at the standard state is given in Table 1. In Table 2 the concentrations of the anions in plasma and of the kations and anions in the erythrocytes as calculated with our model as well as the results calculated by others (13, 63) are compared with experimental values reported in the literature. In Table 3 the osmolality of plasma and erythrocytes as calculated with our model as well as the results as calculated by others (13) are compared with experimental values reported in the literature. With the standard state as the boundary condition, a variation of one or more variables induces changes in the equilibrium state. If the amount of CO_2 is changed, while keeping the amounts of all other substances constant, the resulting changes are those of the titration of blood with CO_2. Thus a bufferline of blood is obtained (Fig. 6A). Rispens (59) found the slope of this bufferline to be -30.7 slyke (mmol·pHu^{-1}) at $c_{b,Hb} = 150$ g·l^{-1}. Our model gives a value of -28.3 slyke. However, this value is in good agreement with the slope of -28.2 slyke which can be derived from figure 12 in reference 72.

In calculating the composition of venous blood it was assumed that the respiratory quotient is 0.83 and venous $pCO_2 = 6.13$ kPa (46 mmHg). The results of this calculation are given in Table 4, together with the values of the considered quantities in the standard state and with the difference between the two states. Bagott (8) reconsidered the values by Dill *et al.* (21) with other parameters for the carbamate equation and obtained results which are in good agreement with our values.

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Table 1. Adopted standard state of human blood

Quantity	Plasma	Erythrocytes	Blood	Unit
		Input values		
Na^+	153			$mmol \cdot l^{-1}$
Protein	70			$g \cdot l^{-1}$
Hemoglobin		333	150	$g \cdot l^{-1}$
ATP		1.5		$mmol \cdot l^{-1}$
S_{O_2}		1.0		
pCO_2	5.33	5.33	5.33	kPa
pH	7.410	7.202		
Temperature	310	310	310	K
		Calculated values per liter blood		
K^+		54		mmol
HCO_3^-	13.7	5.3	19.0	mmol
Cl^-	61.0	23.5	84.5	mmol
2,3-DPG		1.76		mmol
Carbamate		1.13		mmol
Dissolved CO_2	0.67	0.47	1.14	mmol
Total CO_2	14.37	6.88	21.25	mmol
Volume	0.550	0.450	1.000	l
H_2O	0.517	0.323	0.840	l
π_{coll}	3.4	106.9		kPa
π	737	737		kPa

Variation of pH is accompanied by variation of anion concentrations, which in turn leads to a change in osmotic pressure and in osmolality of plasma (Fig. 6B). Because of the Donnan distribution the changes are not equally divided between plasma and red cells. This leads to a changing hematocrit with plasma pH (Fig. 6C). The slope of the calculated curve is -0.032 pHu^{-1}. The value found by Reeves (58) is -0.031 ± 0.002.

Titration of blood with CO_2 also gives a change in total CO_2 content. The total amount of CO_2 in blood has been calculated for oxygenated and deoxygenated blood (Fig. 7). The difference between the two curves is the Haldane effect. At $pCO_2 = 5.33$ kPa (40 mmHg) this difference was calculated to be 0.31 mmol$\cdot$l^{-1} in fair agreement with experimental values (72).

At constant pCO_2 and constant intracellular pH the amount of CO_2 bound as carbamate depends on oxygen saturation. With $pCO_2 = 5.33$ kPa (40 mmHg) and $pH_c = 7.2$ Bauer and Schröder (11) have found an increase of 0.081 mol CO_2 per mol Hb when oxygen saturation changes from one to zero, while plasma pH drops by 0.047 pHu. Under these circumstances we calculated an increase of 0.083 mol CO_2 per mol Hb, while plasma pH had to be lowered by 0.037 pHu to keep the intracellular pH constant.

The Donnan distribution of ions and hydrogen activity between plasma and erythrocytes has been much investigated. In our calculations the Donnan ratios

Table 2. Amounts of electrolytes in plasma and erythrocytes (mmol·kg⁻¹ H₂O)

	Plasma		Erythrocytes	
Experimental values	Σ kat	Cl⁻	Σ kat	Cl⁻
Clauvel et al. (16)	164 ± 3 [2]		173 [3]	
Gleichmann et al. (26) [1]	162 ±		170	
Hald et al. (33) [1]	156 [2]		164	
Valberg et al. (76)[1]	164 ± 4		158	
Diem and Lentner (20)	164	108		
Albritton (4)	158	109	167	73
Bernstein (12)	166 [2]	113	153 [3]	79
Dill et al. (21) [1]	164	112	155	75
Erickson et al. (23)	160 [2]	111	189 [3]	74
Funder and Wieth (25)		110		73
Gram (30)		107−109		73−79
Hastings et al. (34)		104−111		73−84
Von Bubnoff and Riecker (15)				73−91
Models	Σ kat	Cl⁻ [4]	Σ kat	Cl⁻
Brodda (13) [1]	161	*116* [5]	177	*90*
Rodeau and Malan (63) [1]	161	114	188	*74*
Our model [1]	164	*118*	*168*	73

[1] Arterial and arterialized venous blood.

[2] 5 mmol·kg⁻¹ added because Ca^{2+} and Mg^{2+} were not included.

[3] 7 mmol·kg⁻¹ added because Mg^{2+} was not included.

[4] Besides cloride the important anions in plasma are organic acid 6 mmol·kg⁻¹, phosphate 2 mmol·kg⁻¹ and sulfate 1 mmol·kg⁻¹. Rodeau and Malan (63) consider phosphate separately in their model, so their amount of chloride includes 7 mmol·kg⁻¹ of other anions. In the model of Brodda and in our own model all these anions are included in the amount of chloride, which thus has to be diminished by 9 mmol·kg⁻¹ for it to be comparable with the experimental value.

[5] Calculated values in italics.

Table 3. Osmolality of human blood plasma and erythrocytes

	Plasma	Erythrocytes
Experimental values	mosm·kg⁻¹	mosm·kg⁻¹
Diem and Lentner (20)	287	
Funder and Wieth (26)	289	
Hendry (37, 38)	289 ± 4	290 ± 4
Models		
Brodda (13)	*311*[1,2]	*311*[2]
Rodeau and Malan (63)	290	290
Our model	*286* [2]	*286* [2]

[1] Calculated values in italics.

[2] Not included is the contribution of non-electrolytes such as glucose and ureum. When their total amount is taken to be 10 mmol·l⁻¹ of blood, the calculated values have to be augmented by 12 mosm·kg⁻¹. Brodda's value then becomes 323 mosm·kg⁻¹ and our value 298 mosm·kg⁻¹.

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Table 4. Calculated veno-arterial differences in one liter of human blood assuming a respiratory quotient of 0.83 and venous blood with $pCO_2 = 6.13$ kPa (46 mmHg)

| Quantity	Arterial blood			Venous Blood			Veno-arterial Difference			Unit
	Plasma	Erythro-cytes	Blood	Plasma	Erythro-cytes	Blood	Plasma	Erythro-cytes	Blood	
S_{O_2}		1			0.76			−0.24		
pCO_2	5.33	5.33		6.13	6.13		0.80	0.80		kPa
pH	7.410	7.202	$r_{c,p}=0.619$	7.378	7.184	$r_{c,p}=0.638$	−0.032	−0.018		pHu
Total O_2		9.31	9.31		7.07	7.07		−2.23	−2.23	mmol
Total CO_2	14.36	6.89	21.25	15.31	7.81	23.11	0.95	0.92	1.86	mmol
Dissolved CO_2	0.67	0.47	1.14	0.77	0.54	1.31	0.10	0.07	0.17	mmol
Carbamate		1.13	1.13		1.42	1.42		0.29	0.29	mmol
HCO_3^-	13.69	5.29	18.98	14.53	5.85	20.38	0.84	0.56	1.40	mmol
Cl^-	60.90	23.58	84.48	60.20	24.28	84.48	−0.70	0.70		mmol
Charge of protein	9.52			9.39			−0.13			mmol
Charge of hemoglobin		15.47			14.24			−1.23		mmol
Charge of DPG		7.93			7.90			−0.03		mmol
Charge of ATP		1.74			1.73			−0.01		mmol
Volume	0.550	0.450	1.000	0.548	0.452	1.000	−0.002	0.002		l
Water	0.517	0.323	0.840	0.515	0.325	0.840	−0.002	0.002		l
Osmolality	286.1			287.5			1.4			mosm·kg^{-1}
Colloid osmotic pressure	3.44	106.9		3.46	105.6		0.02	−1.3		kPa
Total osmotic pressure	736.8	736.8		740.6	740.6		3.8	3.8		kPa
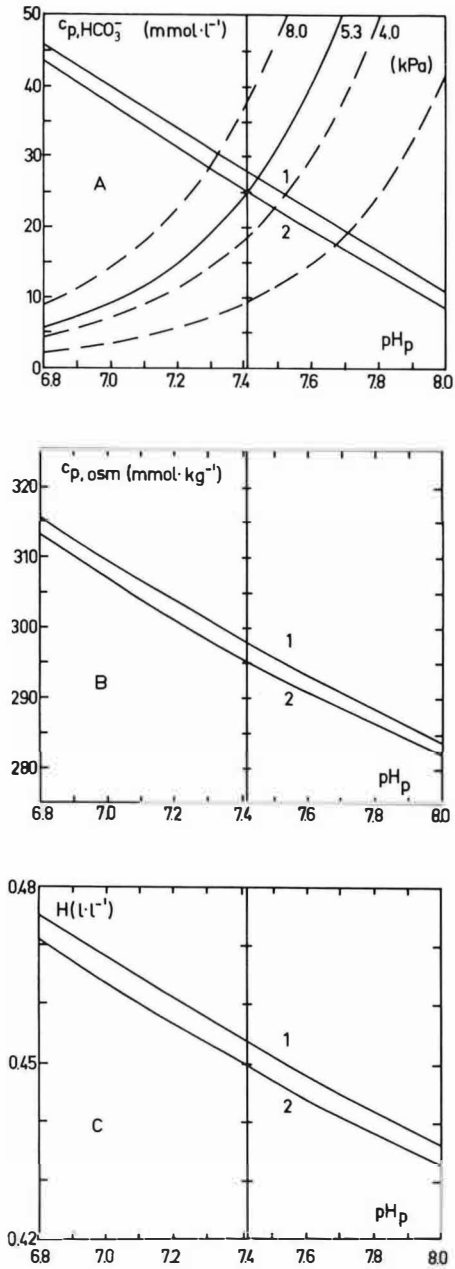
Fig. 6. Calculated relations between some variables and plasma pH when blood is titrated with CO_2; curves 1: oxygen saturation $S_{O_2}=0$; curves 2: oxygen saturation $S_{O_2}=1$. A. Plasma bicarbonate (c_{p,HCO_3^-}). B. Plasma osmolality ($c_{p,osm}$), 10 mmol·kg^{-1} added for glucose and ureum. C. Relative cell volume (hematocrit H).
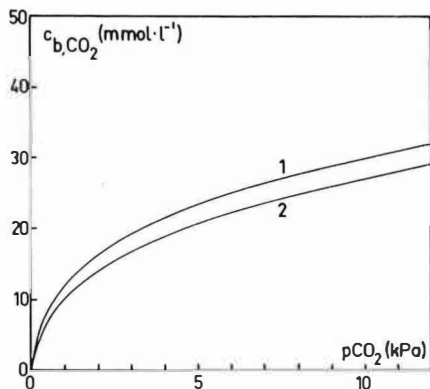
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Fig. 7. Total amount of CO_2 per liter blood (c_{b,CO_2}) plotted against pCO_2, $c_{b,Hb} = 150$ g$\cdot$l^{-1}; curve 1: $S_{O_2} = 0$, curve 2: $S_{O_2} = 1$.

of Cl^-, HCO_3^- and H^+ were supposed to equal (25). The calculated Donnan ratio is given in Fig. 8, together with relations taken from the literature.

The Donnan ratio and the hematocrit of oxygenated blood were calculated over the temperature range of 25° to 40°C under two supposed regimens (Fig. 9). It is clear that the temperature induced changes are less disturbing when the total CO_2 content of the blood is kept constant. This agrees with the experimental results of Reeves (58), who has found that in blood with constant total CO_2 content hematocrit and Donnan ratio hardly change with temperature. However, with constant CO_2 content of the blood the calculated rate of change of pH with temperature is only -0.010 pHu$\cdot$K^{-1} in contrast with experimental values of -0.015 pHu$\cdot$K^{-1} (7) and -0.0144 pHu$\cdot$K^{-1} (1). Furthermore, when $T = 300$ K the calculated pCO_2 is 3.69 kPa (27.7 mmHg), while the reported value is 3.44 kPa (25.8 mmHg) (52). The calculated results depend on the value used for the rate of change of the iso-electric pH of deoxygenated hemoglobin with temperature for which -0.0122 K^{-1} was taken (5). When we changed this value to -0.018 K^{-1} the calculated rate of change of blood pH with temperature was -0.0144 pHu$\cdot$K^{-1} and pCO_2 3.34 kPa (25.1 mmHg) at 300.15 K.

Van Slyke and Sendroy (78) have constructed a nomogram with which the ratio $c_{p,CO_2}/c_{b,CO_2}$ as depending on pH and S_{O_2} can be found. McHardy (50) has derived an empirical relation from values found with this nomogram. Rispens (59) has modified this relation to include temperature. Fig. 10 shows McHardy's relation together with the curves representing the ratio as calculated with the model. Within the range where Van Slyke and Sendroy checked the nomogram experimentally ($7.2 < pH_p < 7.7$), the agreement of the calculated values with those of McHardy lies within 1%.

DISCUSSION

We have tried to derive the acid-base properties of human blood from the relevant physico-chemical properties of its constituents. Until about fifteen

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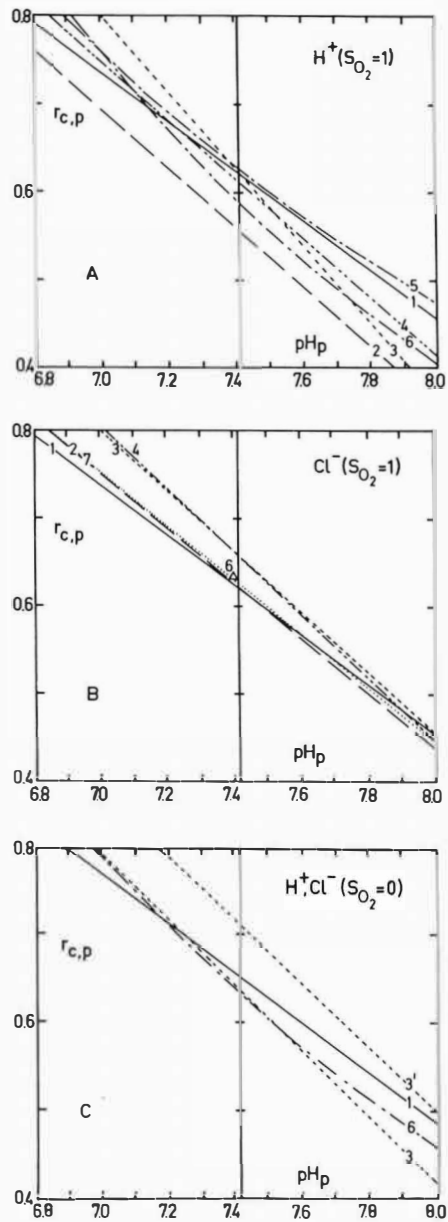
Fig. 8. Donnan ratios ($r_{c,p}$) as calculated for the titration of blood with CO_2 compared with relations from the literature; curves 1: relations as calculated with the help of our model, curves 2: Bromberg *et al.* (14), curves 3: Fitzsimons and Sendroy (24), curves 4: Funder and Wieth (25), curve 5: Hilpert *et al.* (40), curves 6: Takano *et al.* (74), curve 7: Reeves (58). A. Donnan ratio of hydrogen ion activity in oxygenated blood plotted against plasma pH. B. Donnan ratio of chloride anion concentration in oxygenated blood plotted against plasma pH. C. Donnan ratio of hydrogen ion activity (curves 1, 3, 6) and chloride anion concentration (curve 3') in deoxygenated blood.

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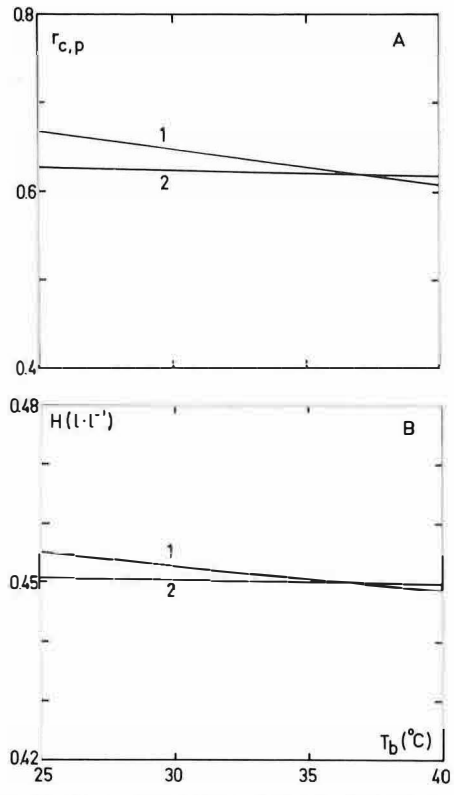
Fig. 9. Donnan ratio ($r_{c,p}$) and hematocrit (H) as calculated over the temperature range 25–40°C under two regimens, curves 1: pCO_2 is kept constant at 5.33 kPa (40 mmHg), curves 2: total CO_2 content kept constant at 21.2 mmol·l^{-1}. A. Donnan ratio plotted against temperature. B. Hematocrit plotted against temperature.
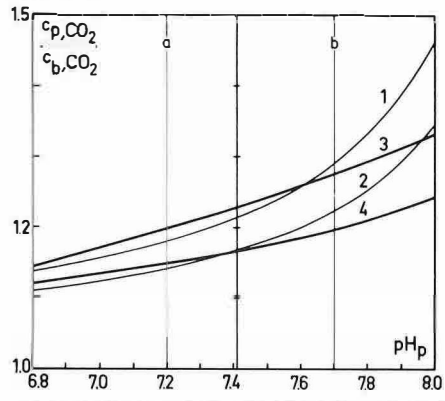
Fig. 10. Ratio of plasma total CO_2 concentration to blood CO_2 concentration plotted against plasma pH. Curves 1 and 3: oxygenated blood, curves 2 and 4: deoxygenated blood. Thin lines according to McHardy (50), thick lines as calculated with the help of our model. The range of pH over which the determinations were carried out, and upon which the McHardy formula is based, lies between a and b.

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years ago acid-base models were presented as nomograms in which the relations between the variables are given in a graphical way (36, 73, 78, 80). Thereafter, more complex models have been published which necessitated the use of a computer. Examples are the models of Lloyd and Michel (48), Roos and Thomas (64), Dell and Winters (17), Rispens (59), Thomas (75), Siggaard-Andersen (72), and more recently Brodda (13) and Rodeau and Malan (63). In two of them (17, 64) blood is only a part of the models which describe acid-base behaviour of blood in contact with interstitial fluid. The models of blood as given by Dell and Winters (17), Roos and Thomas (64), and Thomas (75) are not only based on the properties of the basic constituents of blood but also on experimentally determined relations between some subsystems. Some variables are assumed to be constant in these models.

In their model Rodeau and Malan (63) have used quite a different approach. They relate the amount of imidazole groups of proteins to the buffer power of
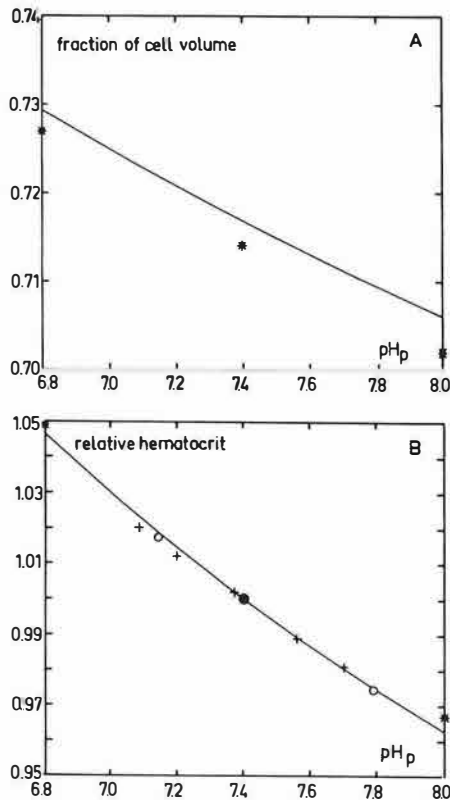
Fig. 11. A. Fraction of cell volume occupied by cell water plotted against plasma pH when blood is titrated with CO_2. Drawn line as calculated with the help of our model; asterisks: values given by Murphy (51). B. Relation between relative hematocrit of blood and plasma pH when blood is titrated with CO_2. Drawn line as calculated with the help of our model; asteriks: values given by Murphy (51), open circles: Dill *et al.* (21), crosses: Reeves (58).

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proteins and do not include the influence of oxygenation and carbamate formation on the titration curve of hemoglobin. Oxygen saturation in their model has a fixed value of one. Carbamate as a factor influencing total CO_2 content of blood is also neglected. In contrast to the other models they include bivalent ions as such. In all other models only the charge contribution of these ions is considered. Rodeau and Malan suppose that plasma has an osmotic coefficient of one and they include 2,3-DPG, ATP and other phosphorylated compounds as buffers.

Our model is in the same line of reasoning as the models of Lloyd and Michel (48) and Brodda (13). In these models the acid-base properties of blood are as far as possible derived from the properties of the constituents. The main difference between our model and the other ones is that we use a more detailed description of the titration curve of hemoglobin in which the effects of oxygenation and carbamate formation have been included. This became possible through results achieved and published in recent years. Another important difference with the other models is the relation we use to describe the osmotic pressure caused by the hemoglobin in erythrocytes. Yet another, but less important difference was the use of an osmotic coefficient for the plasma protein. In the model we have also included 2,3-DPG and ATP as buffers inside the erythrocyte. In this way we got rid of the ''non-diffusible, non-buffer anions'', which were needed to balance electric charges and osmotic forces in the models of Lloyd and Michel (48), and Rispens (59).

The use of a different osmotic pressure relation for hemoglobin turned out to be essential to get results that are consistent with experimental findings. Results that are directly dependent on this relation are the amounts of kations and chloride in the erythrocyte as calculated for the standard state (Table 2) and the variations of cell water and hematocrit with plasma pH as given in Fig. 11.

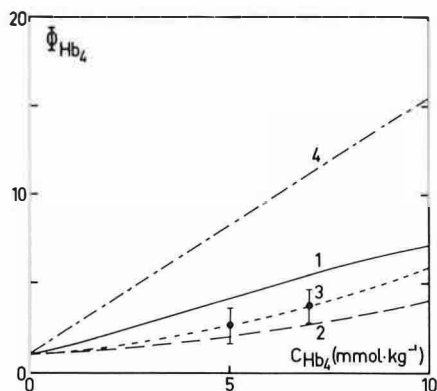Fig. 12. Osmotic coefficient of hemoglobin (ϕ_{Hb_4}) plotted against concentration (c_{Hb_4}); curve 1: as used in our model *i.e.* equal to that of albumin according to Landis and Pappenheimer (46), curve 2: according to Dick and Lowenstein (19), curve 3: calculated with values given by McConaghey and Maizels (49), standard deviation indicated by vertical bars, curve 4: as found by Savitz *et al.* (70).

There is a good agreement between the calculated relations and the experimental findings from the literature. We are aware of the fact that in the way we calculate the osmotic pressure of hemoglobin, generally accepted experimental values are put aside. However, as indicated in the derivation, the relation used is not completely devoid of a physical basis. The multiple interactions in which hemoglobin is involved give rise to many pitfalls in the measurement of the osmotic pressure, some of which were not known in the time of Adair (2, 3). Albumin does not have this drawback to the same extent and is a spherical protein of about the same molecular mass as hemoglobin. So it is rather unlikely that their osmotic coefficients will be very different. The various findings of the osmotic coefficient of hemoglobin and the one assumed by us are given in Fig. 12. Although the results obtained with the presented model should not be seen as proof that the supposed osmotic coefficient of hemoglobin is correct, at least the requirement of Occam's razor is met because a minimal number of assumed essentials makes the model give results which cover the acid-base properties of blood to a remarkable degree.

CONCLUSION

The acid-base and related interactions of blood are fairly well described by this model. The consequences of titration with CO_2 have been presented here. However, the results of titration with HCl and NaOH can also be calculated with the help of this model, as also the changes induced by variation of S_{O_2}, hemoglobin concentration, plasma protein concentration and other parameters. The model is a means to provide insight into the way the variables are related and to check whether the properties of particular constituents fit in with the acid-base properties of blood as they are known from experiments. We find a difference between the osmotic properties of hemoglobin in solution as given in the literature and the properties needed to complete the model in such a way that it gives results that are consistent with experimental findings.

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CHAPTER III

HUMAN WHOLE BLOOD OXYGEN AFFINITY: H$^+$ AND CO$_2$
BOHR AND HALDANE FACTORS CALCULATED FOR
ERYTHROCYTE CONTENT AND BLOOD

Human whole-blood oxygen affinity: H^+ and CO_2 bohr and haldane factors calculated for erythrocyte content and blood

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ABSTRACT

Oxygen affinity of hemoglobin is influenced by pH, pCO_2, and temperature. A mathematical description of the mutually dependent influences of pH, pCO_2, and temperature was formulated by Wyman (1964). In this paper an expression is presented based on Wyman's theory and on some assumptions about proton and carbon dioxide binding to hemoglobin. This expression relates the oxygen affinity of hemoglobin to the intra-erythrocytic value of pH, and to pCO_2 and temperature. In addition to this the model of blood we developed earlier (Zock *et al.*, 1980) was used to relate pH in the red cells to that in plasma. As pCO_2 and temperature were assumed to be the same both inside and outside the erythrocytes, this model enabled us to relate the oxygen affinity of hemoglobin inside the erythrocytes to plasma pH, pCO_2, and temperature. From this the bohr and haldane factors of blood were calculated. A comparison of the values calculated with those mentioned in the literature concerning whole blood shows good agreement.

INTRODUCTION

In a previous paper (33) we presented a set of equations modelling some physico-chemical properties of blood. In that model the oxygen saturation of hemoglobin was one of the variables to which a certain value had to be assigned. However, the normal situation in the body is that the oxygen saturation of hemoglobin is determined by several quantities, of which oxygen tension (pO_2) is the main one. Quantities with a significant influence on the oxygen affinity of hemoglobin are pH, carbon dioxide tension (pCO_2), 2,3-DPG concentration in the erythrocytes and temperature. To bring the model closer to reality, we have added equations relating the oxygen saturation (sO_2) of human hemoglobin to pO_2, pH, pCO_2, and temperature. This

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extended model was used to calculate the oxygen affinity of hemoglobin as the pO_2 at $sO_2 = 0.5$ (p_{50}) in relation to plasma pH, pCO_2, and temperature. The results thus obtained can be compared with those obtained by measuring oxygen affinity in whole blood. The model is therefore open to experimental verification.

Research concerning hemoglobin roughly falls apart into two approaches, the biochemical and the physiological. In the biochemical approach hemoglobin is regarded as a special protein and is studied under conditions which are for the greater part outside the normal, physiological range. This is, of course, a necessity because for many of the properties the physiological range of conditions is too narrow to allow reliable conclusions to be made. Physiological research, on the other hand, concerns the transport capabilities of hemoglobin in blood and experiments are done with blood under physiological conditions. Under these circumstances the hemoglobin solution is locked up inside the erythrocytes, so that information concerning the direct environment of the hemoglobin molecules is more difficult to obtain than in the biochemical studies. This applies mainly to intracellular pH, as the 2,3-DPG concentration changes more slowly and can be determined afterwards. Temperature, pO_2, and pCO_2 can be considered to be the same as outside the erythrocytes. Because the intracellular pH is not uniquely related to plasma conditions, referring to extracellular values, as is often done, is prone to errors.

The biochemical approach has resulted in a more or less detailed knowledge of the binding of oxygen, protons, carbon dioxide and 2,3-DPG to the hemoglobin molecule. Combination with chemical thermodynamics has led to mathematical formulations of the mutual interactions in the binding of these ligands (31). With this theory and using characteristic values for the ligand binding, it is possible to calculate changes in the oxygen affinity of hemoglobin caused by changes in intracellular pH, pCO_2 and temperature. The appropriate detailed quantitative knowledge of the mutual interactions of 2,3-DPG with the other ligands is at present still lacking. This makes it impossible to calculate the effects of changes in 2,3-DPG concentration.

On the basis of the above, the assigned value of sO_2 in the model was replaced by expressions relating sO_2 to intracellular pO_2, pH, pCO_2 and temperature. A normal 2,3-DPG concentration inside the erythrocytes was assumed throughout.

The model, which has been described earlier (33), provides the relation between the compositions of the intra-erythrocytic compartment and the blood plasma compartment. Changes in the oxygen affinity of hemoglobin resulting from changes inside the erythrocytes can thus be related to concomitant changes in the plasma composition. The calculated values are compared with results of measurements in whole blood as given in the literature.

THE OXYGEN AFFINITY OF HEMOGLOBIN

The reversible binding of oxygen to hemoglobin is influenced by several quantities defining the state of the solution. Quantities exerting major in-

fluences are pH, pCO_2, 2,3-DPG concentration in the solution, and temperature. These will be called affinity modifiers. Other less powerful influences are ionic strength and the concentrations of certain ions. We shall assume these to have normal physiological values throughout. When the modifiers are kept at constant values, a unique relation exists between pO_2 and sO_2 of hemoglobin. From its graphical representation, this relation is commonly referred to as the oxygen dissociation curve (ODC) or the oxygen equilibrium curve (OEC), the term preferred here. The effect of the modifiers on the oxygen affinity of hemoglobin is usually measured through the amount of change induced with respect to the OEC of hemoglobin under standard conditions.

The influence of the modifiers mainly consists of a shift of the OEC along the log (pO_2) axis with only minor or no changes in its shape. The shape seems to be particularly indifferent to changes in temperature (35). Invariance of the shape means that the OEC is completely defined by only one parameter, for which p_{50} is the generally accepted quantity. Under the assumption of invariance of shape, the description of the influence of the affinity modifiers on the OEC is reduced to the description of their influence on p_{50} only. However, the modifiers with the exception of temperature also slightly change the shape of the OEC. As a consequence of this, p_{50} is theoretically not a good measure of the oxygen affinity of hemoglobin. Instead, Wyman (31) has defined a more general measure of affinity for ligands bound to large molecules like hemoglobin which he calls the median ligand activity. For the reversible binding of oxygen to hemoglobin the median ligand activity (p_m) is defined by

$$(1) \qquad \int_0^{p_m} sO_2 \, d(\log \, (pO_2)) = \int_{p_m}^{\infty} (1 - sO_2) \, d(\log \, (pO_2))$$

in which the sO_2 is a function of pO_2 ranging from 0 to 1 and log (pO_2) is the logarithm of pO_2 to the base 10.

From this, it is clear that $p_{50} = p_m$ if the relation between sO_2 and log (pO_2) would be symmetrical around p_{50} in the sense that

$$(2) \qquad sO_2\left(pO_2 = \frac{1}{a} \cdot p_{50}\right) = 1 - sO_2(pO_2 = a \cdot p_{50})$$

In that case the hill plot, *i.e.* log $(sO_2/(1 - sO_2))$ *versus* log (pO_2), would also be symmetrical around p_{50}. In practice, the difference between p_m and p_{50} is small and as long as no changes in shape occur the change in log (p_m) is equal to that in log (p_{50}). Therefore, in this paper derivations that are valid for p_m will be considered applicable to p_{50} as well.
will be considered applicable to p_{50} as well.

The linkage equations derived by Wyman (31) relate the change in affinity of large molecules such as hemoglobin for a certain ligand under the influence of a change in the activity of a second ligand to the amount of the latter ligand that is bound to the molecule. The interactions between oxygen and the other ligands arise through the influence which the combination of hemoglobin with

oxygen exerts on the equilibrium constants of the reactions of the other ligands with hemoglobin. Temperature directly influences the equilibrium constants.

According to this, the relation between p_m as a measure of the oxygen affinity of hemoglobin and pH is

(3) $$\frac{\partial \log (p_m(pH, pCO_2, T))}{\partial pH} = -\Delta z(pH, pCO_2, T)$$

in which Δz is the amount of protons released per hemoglobin monomer when hemoglobin passes from the deoxygenated state Hb_4 to the completely oxygenated state Hb_4O_8, while the modifiers remain constant. It was assumed that this amount is equal to the increase in the net negative charge of the hemoglobin monomer:

(4) $$\Delta z(pH, pCO_2, T) = z_d(pH, pCO_2, T) - z_o(pH, pCO_2, T)$$

where the indices "d" and "o" refer to deoxyhemoglobin and oxyhemoglobin, respectively.

In the same way, the relation between the amount of CO_2 bound to hemoglobin (as carbaminohemoglobin) and p_m is

(5) $$\frac{\partial \log (p_m(pH, pCO_2, T))}{\partial \log (pCO_2)} = \Delta Z(pH, pCO_2, T)$$

in which ΔZ is the amount of CO_2 released per hemoglobin monomer when hemoglobin passes from the deoxygenated to the completely oxygenated state and the modifiers are kept constant.

At $pCO_2 = 0$ the protons released on oxygenation originate from the acidic and alkaline bohr groups only. Thus

(6) $$\Delta z = \Delta z_A + \Delta z_B$$

in which Δz_A refers to the acidic and Δz_B to the alkaline groups and from which the arguments pH, pCO_2, and T have been omitted. In line with the original proposal of Wyman (30), it was assumed that a hemoglobin monomer has one acidic bohr group, so that Δz_A is equal to the difference between the saturation of the acidic group with protons in the deoxygenated state (sH_{ad}^+) and the saturation in the oxygenated state (sH_{ao}^+). Saturation ranges from zero to one. Thus:

(7) $$\Delta z_A = sH_{ad}^+ - sH_{ao}^+$$

This can conveniently be written as

(8) $$\Delta z_A = [sH_{ax}^+]_{x=o}^{x=d}$$

in which the state variable x can take one of the two values "d" and "o". The degree of ionization of an acidic group equals one minus the saturation with protons. Consequently,

(9) $$(1 - sH_{ax}^+) = \frac{1}{1 + 10^{pK_{ax} - pH}}$$

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Because the pK values of the acidic group (pK_{ax}) depend on the state of oxygenation the same holds for the saturation with protons. In deoxyhemoglobin $pK_{ux} = pK_{ad}$, in oxyhemoglobin $pK_{ax} = pK_{ao}$. The equations 7 and 8 can be written as

$$(10) \qquad \Delta z_A = -(1 - sH^+_{ad}) + (1 - sH^+_{ao}) \equiv [-(1 - sH^+_{ax})]^{x=d}_{x=o}$$

The difference in charge caused by the alkaline bohr groups Δz_B, is the result of changes in saturation with protons of several amino groups on a hemoglobin molecule when hemoglobin passes from the deoxygenated to the oxygenated state (16). This change in proton saturation is brought about by the changes in pK of these groups that accompany changes in the state of oxygenation. Some of the CO_2 is bound to hemoglobin by combination with some of the alkaline bohr groups. In the model we have approached this by assuming that two alkaline bohr groups reside on each hemoglobin monomer: one that does not combine with CO_2 (indicated as b), and one that does (indicated as z). At $pCO_2 = 0$ all of the latter groups contribute to the alkaline bohr effect. Accordingly,

$$(11) \qquad \Delta z_B = \Delta sH^+_b + \Delta sH^+_z$$

which can be written as

$$(12) \qquad \Delta sH^+_b = sH^+_{bd} - sH^+_{bo} \equiv [sH^+_{bx}]^{x=d}_{x=o}$$

and

$$(13) \qquad \Delta sH^+_z = [sH^+_{zx}]^{x=d}_{x=o}$$

In alkaline groups ionization is caused by proton binding, so that the degree of ionization is equal to the saturation with protons. Thus

$$(14) \qquad sH^+_{bx} = \frac{1}{1 + 10^{pH - pK_{bx}}}$$

and

$$(15) \qquad sH^+_{zx} = \frac{1}{1 + 10^{pH - pK_{zx}}}$$

The values of both pK's as well as their changes with temperature depend on the state of oxygenation.

At $pCO_2 = 0$ the change in the amount of bound protons as caused by oxygenation is described by

$$(16) \qquad \Delta z = [-(1 - sH^+_{ax}) + sH^+_{bx} + sH^+_{zx}]^{x=d}_{x=o}$$

According to equation 3, changes in p_m caused by changes in pH are calculated by integration of Δz with respect to pH. Integration from pH_1 to pH_2 using equations 9, 14 and 15 leads to

$$(17) \qquad \left\{ \begin{aligned} \Delta \log (p_m) = [[\log \{(1 + 10^{pH - pK_{ax}}) \cdot (1 + 10^{pK_{bx} - pH}) \cdot \\ \cdot (1 + 10^{pK_{zx} - pH})\}]^{x=d}_{x=o}]^{pH_2}_{pH_1} \end{aligned} \right.$$

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In this expression the order of the substitution of x and pH can be interchanged. This corresponds with the notion that when a combination of a reversible oxygenation and a reversible change in pH occurs, the change in z is the same irrespective of the order of the oxygenation and the change in pH. Substitution of x and pH gives four terms, each with one of the possible combinations of x and pH. The combinations (d, pH_2) and (o, pH_1) take a positive sign, the other two a negative one.

When CO_2 is present in the solution, the effect of carbamino formation must be taken into account. This effect is three-fold. Firstly, CO_2 is a ligand and changes p_m according to equation 5. Secondly, with the binding of CO_2, alkaline bohr groups disappear. Thirdly, the carbamino groups formed with the binding of CO_2 are strongly acidic; they contribute to the amount of protons released from hemoglobin when it passes from the deoxygenated to the completely oxygenated state, because carbamino formation strongly depends on oxygenation.

Carbamino-hemoglobin is formed by the reversible combination of CO_2 with certain aminogroups on the hemoglobin molecule according to

$$(18) \qquad HbNH_3^+ \leftrightarrows HbNH_2 + H^+ \qquad : pK_{zx}$$

$$(19) \qquad HbNH_2 + CO_2 \leftrightarrows HbNHCOO^- + H^+ \qquad : pK_{cx}$$

It is a generally accepted assumption that the carbamino groups on hemoglobin are completely dissociated. Therefore, the amount of protons released on carbamino formation equals, according to equation 19, the amount of CO_2 bound. In the presence of CO_2, Δz thus contains additional terms to account for the latter two of the three effects above mentioned. It follows from these equilibria that the ratio Z_x between the amount of carbamino-hemoglobin and the total amount of hemoglobin monomer is given by

$$(20) \qquad Z_x = \frac{S \cdot pCO_2}{S \cdot pCO_2 + 10^{pK_{cx} - pH} \cdot (1 + 10^{pK_{zx} - pH})}$$

in which S is the solubility of CO_2 in the hemoglobin solution. The ratio Z_x is different for deoxyhemoglobin and oxyhemoglobin. This is because pK_{cx} and pK_{zx} have different values for deoxyhemoglobin and oxyhemoglobin. The amount of CO_2 released per hemoglobin monomer on oxygenation at constant pH, pCO_2, and T is given by

$$(21) \qquad \Delta Z = Z_d - Z_o \equiv [Z_x]_{x=o}^{x=d}$$

As already mentioned, the combination of CO_2 with hemoglobin causes the disappearance of one alkaline bohr group. When a fraction Z_x of the available groups is occupied by CO_2, $(1 - Z_x)$ groups remain. It is assumed that the saturation with protons of the CO_2 binding amino groups that do not carry

CO_2 is unchanged. The amount of protons per hemoglobin monomer is thus $(1 - Z_x) \cdot sH_{zx}^+$. The expression for z_B then becomes:

(22) $\quad \Delta z_B = [sH_{bx}^+ + (1 - Z_x) \cdot sH_{zx}^+ - Z_x]_{x=o}^{x=d}$

and therefore

(23) $\quad \Delta z = [-(1 - sH_{ax}^+) + sH_{bx}^+ + (1 - Z_x) \cdot sH_{zx}^+ - Z_x]_{x=o}^{x=d}$

In accordance with equation 5, the effect of CO_2 proper on p_m can be obtained by integrating equation 21 with respect to $\log(pCO_2)$. Integration from $pCO_{2,1}$ to $pCO_{2,2}$ gives

(24) $\quad \Delta \log (p_m) = \left[\left[\log \left\{ 1 + \dfrac{S \cdot pCO_2}{10^{pK_{cx} - pH} \cdot (1 + 10^{pK_{zx} - pH})} \right\} \right]_{x=o}^{x=d} \right]_{pCO_{2,1}}^{pCO_{2,2}}$

Combination of this equation with equation 17 gives the expression for the case where changes in pH and pCO_2 occur simultaneously:

(25) $\quad \left\{ \begin{aligned} \Delta \log (p_m) &= \left[\left[\log \left\{ (1 + 10^{pH - pK_{ax}}) \cdot (1 + 10^{pK_{bx} - pH}) \cdot (1 + 10^{pK_{zx} - pH}) \cdot \right. \right. \right. \\ &\quad \left. \left. \left. \cdot \left(1 + \dfrac{S \cdot pCO_2}{10^{pK_{cx} - pH} \cdot (1 + 10^{pK_{zx} - pH})} \right) \right\} \right]_{x=o}^{x=d} \right]_{pH_1, pCO_{2,1}}^{pH_2, pCO_{2,2}} \end{aligned} \right.$

Consequently,

(26) $\quad \left\{ \begin{aligned} \log (p_m) &= constant + \left[\log \left\{ (1 + 10^{pH - pK_{ax}}) \cdot (1 + 10^{pK_{bx} - pH}) \cdot \right. \right. \\ &\quad \left. \left. \cdot (1 + 10^{pK_{zx} - pH}) \cdot \left(1 + \dfrac{S \cdot pCO_2}{10^{pK_{cx} - pH} \cdot (1 + 10^{pK_{zx} - pH})} \right) \right\} \right]_{x=o}^{x=d} \end{aligned} \right.$

The value of the constant is the logarithm of p_m in some reference state with the reference values pH_r, $pCO_{2,r}$, and T_r, minus the value of the function between the square brackets in this reference state. The part of the right hand side of equation 26 with the square brackets will be henceforth referred to as P.

Changes in p_m occurring with changes in temperature (T) are related to the apparent heat of combination of oxygen with hemoglobin according to

(27) $\quad \dfrac{\partial \log (p_m)}{\partial (1/T)} = \dfrac{\Delta Q}{R \cdot \ln (10)}$

in which ΔQ is the total heat of the reaction and R is the universal gas constant 8.314 J/mol $\cdot$ K. The total heat is the result of the binding of oxygen and the concomitant changes in ionization and in the amounts of other substances bound to hemoglobin:

(28) $\quad \Delta Q = \Delta Q_i + \Delta Q_{O_2}$

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in which ΔQ_{O_2} is the heat of the binding of oxygen and ΔQ_i is the heat of the accompanying ionizations including carbon dioxide binding. Besides that of oxygen ΔQ_{O_2} was assumed to include the heat of combination with hemoglobin of the substances not accounted for in ΔQ_i like *e.g.* 2,3-DPG.

In the equation 26, temperature is implicitly present in the temperature dependence of the various pK's and in the solubility of CO_2. With these dependences inserted, the function P is a function of temperature as well, and can be used to calculate that part of ΔQ that is caused by changes in ionization and carbon dioxide binding with temperature:

$$(29) \qquad \Delta Q_i = R \cdot \ln(10) \cdot \frac{\partial P}{\partial(1/T)}$$

According to this

$$(30) \quad \left\{ \begin{aligned} \Delta Q_i = R \cdot \ln(10) \cdot \Bigg[&-(1 - sH_{ax}^+) \cdot \frac{dpK_{ax}}{d(1/T)} + sH_{bx}^+ \cdot \frac{dpK_{bx}}{d(1/T)} + \\ &+ (1 - Z_x) \cdot sH_{zx}^+ \cdot \frac{dpK_{zx}}{d(1/T)} + Z_x \cdot \left(\frac{1}{S \cdot \ln(10)} \cdot \frac{dS}{d(1/T)} - \frac{dpK_{cx}}{d(1/T)} \right) \Bigg]_{x=o}^{x=d} \end{aligned} \right.$$

From the equations 27 and 28 it follows that

$$(31) \qquad \frac{\partial \log(p_m)}{\partial(1/T)} = \frac{1}{R \cdot \ln(10)} \cdot (\Delta Q_{O_2} + \Delta Q_i)$$

Over a restricted range of temperature, ΔQ_{O_2} can be taken to be constant. Because the combination of oxygen with hemoglobin is exothermic, ΔQ_{O_2} is negative. A part of the energy of the reaction is used in the change of ionization; this energy ΔQ_i is positive because energy is used up to increase ionization. The total heat of ionization is the weighted sum of the heats of ionization of the various groups. The term ΔQ_{O_2} represents that part of the heat of oxygenation which is not accounted for in ΔQ_i. This is the reaction with oxygen itself and, if present, that with 2,3-DPG. Possibly, the binding of other ions also contributes to ΔQ_{O_2} to a lesser degree. From the known dependence of p_m on temperature it follows that all these heats add up to -43 kJ/mol at $pH = 7.4$, $pCO_2 = 5.3$ kPa, and $T = 310$ K (35). With the adopted temperature dependencies of pK and S, the heat of ionization ΔQ_i calculated according to equation 30 amounts to about 51 kJ/mol. In order to obtain a temperature dependence of p_m in accordance with experimental values ΔQ_{O_2} was taken to be -94 kJ/mol. A comparison with known thermodynamic values is beyond the scope of this paper.

The relation giving p_m including its temperature dependence follows from the integration of equation 31 with respect to $(1/T)$. Considering that ΔQ_i is $R \cdot \ln(10)$ times the derivative of P, this gives

$$(32) \qquad \log(p_m) = \log(p_{mr}) + \left[\frac{\Delta Q_{O_2}}{R \cdot T \cdot \ln(10)} + P \right]_{(pH_r, pCO_{2,r}, T_r)}^{(pH, pCO_2, T)}$$

in which p_{mr} is the median oxygen affinity at reference conditions (pH_r, $pCO_{2,r}$, T_r). This function represents the oxygen affinity of hemoglobin dependent upon pH, pCO_2, and T under the restrictions imposed by the assumptions adopted.

To be able to compare values from the literature with those calculated with the relations derived here, $\log(p_m)$ can be expanded in a taylor series about the reference point:

$$
(33) \quad \left\{
\begin{aligned}
\log(p_m(pH, pCO_2, T)) &= \log(p_m(pH_r, pCO_{2,r}, T_r)) + \\
&\quad + A \cdot \Delta pH + B \cdot \Delta \log(pCO_2) + \\
&\quad + C \cdot \Delta(1/T) + D \cdot \Delta pH \cdot \Delta \log(pCO_2) + \\
&\quad + E \cdot \Delta \log(pCO_2) \cdot \Delta(1/T) + \\
&\quad + F \cdot \Delta(1/T) \cdot \Delta pH + \ldots
\end{aligned}
\right.
$$

in which $\Delta pH = pH - pH_r$, $\Delta \log(pCO_2) = \log(pCO_2/pCO_{2,r})$, and $\Delta(1/T) = 1/T - 1/T_r$. The coefficients of ΔpH, $\Delta \log(pCO_2)$, and $\Delta(1/T)$ are equal to the first partial derivatives of equation 32:

$$
(34) \quad \left\{
\begin{aligned}
A &= \frac{\partial \log(p_m)}{\partial pH} = -\Delta z \\
B &= \frac{\partial \log(p_m)}{\partial \log(pCO_2)} = \Delta Z \\
C &= \frac{\partial \log(p_m)}{\partial(1/T)} = \frac{\Delta Q}{R \cdot \ln(10)}
\end{aligned}
\right.
$$

The derivative with respect to pH, A, gives the influence of pH, $i.e.$ H^+ activity, on p_m and is therefore called the proton or H^+ bohr factor. The derivative with respect to $\log(pCO_2)$, B, representing the CO_2 influence on p_m, is accordingly called the CO_2 bohr factor. The second derivatives give the mutual dependencies of the first derivatives. These mutual influences are sometimes called interdependent ligand effects.

Table 1. Values of pK's and their temperature dependence as used in the calculations.

index	deoxyhemoglobin		oxyhemoglobin	
	pK	dpK/d(1/T)	pK	dpK/d(1/T)
a	5.13	− 846	5.60	306
b	7.56	2340	6.65	1224
z	7.30	1500	7.00	1500
c	4.60	− 1828	5.00	− 1828

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In our model they are:

$$
(35) \left\{
\begin{aligned}
D &= \frac{\partial}{\partial \log (p\mathrm{CO_2})} \left(\frac{\partial \log (p_\mathrm{m})}{\partial \mathrm{pH}} \right) = \frac{\partial}{\partial \mathrm{pH}} \left(\frac{\partial \log (p_\mathrm{m})}{\partial \log (p\mathrm{CO_2})} \right) = \\
&= \ln (10) \cdot [Z_x \cdot (1 - Z_x) \cdot (1 + s\mathrm{H}_{zx}^+)]_{x=o}^{x=d} \\
E &= \frac{\partial}{\partial (1/T)} \left(\frac{\partial \log (p_\mathrm{m})}{\partial \log (p\mathrm{CO_2})} \right) = \frac{\partial}{\partial \log (p\mathrm{CO_2})} \left(\frac{\partial \log (p_\mathrm{m})}{\partial (1/T)} \right) = \\
&= \ln (10) \cdot \left[Z_x \cdot (1 - Z_x) \cdot \left(\frac{1}{S \cdot \ln (10)} \cdot \frac{dS}{d(1/T)} - \frac{d\mathrm{p}K_{cx}}{d(1/T)} - s\mathrm{H}_{zx}^+ \cdot \frac{d\mathrm{p}K_{zx}}{d(1/T)} \right) \right]_{x=o}^{x=d} \\
F &= \frac{\partial}{\partial (1/T)} \left(\frac{\partial \log (p_\mathrm{m})}{\partial \mathrm{pH}} \right) = \frac{\partial}{\partial \mathrm{pH}} \left(\frac{\partial \log (p_\mathrm{m})}{\partial (1/T)} \right) = \\
&= - \ln (10) \cdot \left[(1 - s\mathrm{H}_{ax}^+) \cdot s\mathrm{H}_{ax}^+ \cdot \frac{d\mathrm{p}K_{ax}}{d(1/T)} + (1 - s\mathrm{H}_{bx}^+) \cdot s\mathrm{H}_{bx}^+ \cdot \frac{d\mathrm{p}K_{bx}}{d(1/T)} + \right. \\
&\quad + (1 - s\mathrm{H}_{zx}^+) \cdot s\mathrm{H}_{zx}^+ \cdot \frac{d\mathrm{p}K_{zx}}{d(1/T)} + \\
&\quad \left. - Z_x \cdot (1 - Z_x) \cdot \left(\frac{1}{S \cdot \ln (10)} \cdot \frac{d(S)}{d(1/T)} - \frac{d\mathrm{p}K_{cx}}{d(1/T)} - s\mathrm{H}_{zx}^+ \cdot \frac{d\mathrm{p}K_{zx}}{d(1/T)} \right) \right]_{x=o}^{x=d}
\end{aligned}
\right.
$$

Table 1 presents the values of pK's and their temperature dependence as used in the calculations. The values of pK_a and pK_b are as given by Rossi-Bernardi and Roughton (24). The values of pK_c and pK_z are reasonable estimates based on the values given by Klocke (17), Perrella (20), Bauer (5), and those given by Rossi-Bernardi and Roughton (23) based on values of Ferguson (8). The values chosen fulfill the requirement that they comply with experimental values and lead to generally accepted values concerning the bohr effects and carbamino hemoglobin in the deoxy and oxy form as well. The solubility S and other quantities not mentioned here have the same values as in the previous paper (33).

THE OXYGEN EQUILIBRIUM CURVE

The standard OEC of blood is related to the conditions $T = 310$ K, $\mathrm{pH}_p = 7.4$, $p\mathrm{CO_2} = 5.33$ kPa, and a normal 2,3-DPG concentration in the erythrocytes of 0.92 mol 2,3-DPG per mol Hb$_4$. Numerous expressions to describe the OEC have been presented. In 1910 Hill published his well-known equation (11). The Adair equation (2) is based on the four successive steps in the oxygen binding reaction. Recent values of the four association constants are given by Roughton and Severinghaus (24). A number of expressions have been put forward that are not based on a reaction mechanism but are intended to approximate measured curves as closely as possible (10, 15, 18, 26, 28). The equation presented by Lobdell (18) is both simple and readily invertible. This equation, which is used in our model, is

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(36) $$sO_2 = \frac{a \cdot p_r^n + b \cdot p_r^{2n}}{1 + c \cdot p_r^n + b \cdot p_r^{2n}}$$

The inverse equation is

(37) $$p_r = \left\{ \frac{a - c \cdot sO_2 - \sqrt{(a - c \cdot sO_2)^2 - 4 \cdot b \cdot (sO_2 - 1) \cdot sO_2}}{2 \cdot b \cdot (sO_2 - 1)} \right\}^{1/n}$$

in which sO_2 is the oxygen saturation, and p_r is the reduced oxygen tension, *i.e.* pO_2/p_{50}, and a, b, c, and n are constants. Their values are given in the caption of table 2, which gives a comparison between measured values of pO_2 at fixed values of sO_2 (34) and the values of pO_2 resulting from equation 37, using these values for the constants. Calculation of p_m using equation 36 with the values of the constants as given shows p_m to be about 1.06 times p_{50}.

Table 2. Comparison of pO_2 calculated according to Lobdell (18) with values measured (34). Standard conditions pH = 7.4, pCO_2 = 5.33 kPa, and T = 310 K. The values of the parameters used were: n = 1.58, a = 0.355, b = 0.645, and c = 0.355.

sO_2	pO_2 measured (kPa)	pO_2 calculated (kPa)
0.1	1.39	1.37
0.2	2.01	2.00
0.3	2.52	2.52
0.4	3.01	3.01
0.5	3.53	3.53
0.6	4.12	4.12
0.7	4.84	4.84
0.8	5.86	5.87
0.9	7.76	7.77
0.95	10.14	9.98

MODEL

In the model blood was assumed to consist of two compartments, each containing a homogeneous solution. The compartment representing plasma was assumed to contain a solution of NaCl, NaHCO$_3$, and protein in water. The other compartment, representing the red blood cells, was assumed to contain a solution of KCl, KHCO$_3$, ATP, 2,3-DPG, and hemoglobin in water. The two compartments were taken to be separated by a membrane which was assumed to be impermeable to Na$^+$, K$^+$, ATP, 2,3-DPG, hemoglobin, and plasma protein. It was assumed to be permeable to O$_2$, CO$_2$, HCO$_3^-$, Cl$^-$, H$^+$, and water. The two compartments were assumed to be in osmotic equilibrium. These aspects of the model have been described in detail earlier (33). Changes with respect to the earlier version of the model are the slightly different values of some pK's and their temperature dependencies (Table 1).

The present version of the model has been extended with the OEC and the dependence of p_m on pH, pCO_2, and temperature in the hemoglobin solution representing red cell content. A further change is the notion that pK_z belongs

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to an alkaline bohr group that not only binds CO_2 but also contributes to H^+ binding.

The reference value of p_m was taken to be 3.53 kPa at $pH_c = 7.192$, $pCO_2 = 5.33$ kPa, and $T = 310$ K. The 2,3-DPG concentration used resulted from the various conditions that had to be satisfied at the reference state. In the model it amounts to 0.92 mol/mol Hb_4.

CALCULATIONS

The equations derived above allow the calculation of the change in the p_m of human hemoglobin in solution with changes in pH, pCO_2, and temperature in this solution. The results presented were obtained by substituting the values of pH, pCO_2 and T in the relevant equations.

The changes in p_m under the influence of changes in plasma values cannot be calculated by direct substitution in the equations. Instead, the plasma values were used to calculate the intracellular pH at $sO_2 = 0.5$ since temperature and pCO_2 were the same both inside and outside the red cell, these values and the intracellular pH were substituted in equation 32, thus giving p_m under these conditions. From these sets of values the whole blood bohr factors were calculated.

To calculate the whole blood H^+ haldane factor at a chosen pCO_2, plasma pH was controlled by the simulated addition of HCl or NaOH. At the same values of plasma pH, pCO_2, and T, but with $sO_2 = 0$ and $sO_2 = 1$ the amounts of NaOH or HCl needed to keep plasma pH constant were calculated as well. The difference between the amount of HCl and NaOH at $sO_2 = 0$ and that at $sO_2 = 1$, divided by the amount of hemoglobin monomer, gives the whole blood H^+ haldane effect at these plasma conditions, *i.e.* the amount of fixed acid that has to be withdrawn or added to keep plasma pH constant during the oxygenation of blood.

Using the same set of values of pH, pCO_2, and T, with $sO_2 = 0$ and $sO_2 = 1$, the total amounts of CO_2 present in blood were also calculated. The difference between the amount of CO_2 at $sO_2 = 0$ and that at $sO_2 = 1$, divided by the amount of hemoglobin gives the whole blood CO_2 haldane factor.

By taking into consideration only those protons and that CO_2 that bind to hemoglobin, the contributions of hemoglobin to the whole blood H^+ haldane and the whole blood CO_2 haldane factors were calculated, *i.e.* Δz and ΔZ. These values differ from those of a hemoglobin solution because intracellular pH is not constant under these circumstances.

The calculations were made for values of plasma pH ranging from 6.7 to 8.1 in steps of 0.05 pHu at pCO_2 0.16 to 29 kPa in steps of $\log (pCO_2) = 0.25$ for the temperatures 290 to 320 K in steps of 5 K.

The program for these calculations was run on a HP9845A desk top computer. The program was written in BASIC. It contained a number of iterative procedures. By controlled variation of some variables, the values were made to converge to an end state in which all the conditions set by the equations were satisfied to within 0.001 mmol/L.

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In blood the H^+ and CO_2 bohr factors were calculated from the values of log (p_m) at the concomitant values of plasma pH, pCO_2, and temperature. This was done by fitting a taylor series over the 26 neighbouring points using a least square method. The taylor series contained the three first derivatives with respect to plasma pH, log (pCO_2) and $(1/T)$, and three partial second derivatives as coefficients. Only three second derivatives were used because it was assumed that the result of differentiation is independent of the order. The first derivative with respect to pH represents the whole blood H^+ bohr factor, that with respect to log (pCO_2) the whole blood CO_2 bohr factor. The derivative with respect to $(1/T)$ represents the temperature dependence of log (p_m) in whole blood. The second derivatives give the mutual influences of the variables on these factors analogous to those derived for hemoglobin solutions in equation 35.

RESULTS AND DISCUSSION

The discovery of what is now called the bohr effect by Bohr *et al.* (6) concerned the influence of CO_2 on the position of the OEC of blood. This effect can be observed when blood is titrated with CO_2 without controlling pH. In fact, the shift is the result of a combination of two effects, the origins of which lie in the nature of the interactions of hemoglobin with protons and CO_2. Titration of blood with CO_2 liberates protons and CO_2 itself interacts with hemoglobin by forming carbamino-hemoglobin.

The original haldane effect (7) is the complement of the original bohr effect, *i.e.* the influence of the oxygen saturation on the equilibrium concentration of all CO_2 present in blood at a certain pCO_2. This, too, is a combination of two effects. The increased CO_2 content of deoxygenated blood is caused by the decrease in acidity of hemoglobin in additon to the increased CO_2 binding by hemoglobin. The property that these two affinities of hemoglobin change on oxygenation is a thermodynamical necessity complementary to the two bohr effects as was first shown by Adair (1) and further elaborated by Wyman (31). Thus, for hemoglobin the H^+ bohr factor related to the median oxygen tension is equal to the H^+ haldane factor. The same equality applies to the CO_2 bohr and haldane factors. This complementariness is expressed in equations 3 and 5 and underlies the equations derived from them.

At a time that little was known about the structure of hemoglobin, Wyman (30) showed that the titration data of hemoglobin could be explained by the assumption that each hemoglobin monomer has two oxygen-linked titrable groups. These groups were supposed to change their pK's on oxygenation, the pK of one group lying in the acidic range of pH (acid bohr group), the other pK in the alkaline range (alkaline bohr group). It is clear now, that these oxygen-linked groups are not evenly distributed over the four hemoglobin monomers of the Hb_4 molecule and that the bohr effect may involve complex interactions between the four chains as well. It has been shown (16) that most of the alkaline bohr effect can be ascribed to the α-amino part of the N-terminal valines of the α-chains and the imidazole part of the histidines 146 of the

β-chains. In the presence of 2,3-DPG the proton bohr effect increases because the binding of 2,3-DPG includes binding of H^+ (22). The amount of bound 2,3-DPG diminishes on oxygenation. The 2,3-DPG is bound to the α-amino parts of the N-terminal valines of the two β-chains. Because these valines can also bind CO_2, 2,3-DPG and CO_2 are competitive here. These valines do not contribute to the proton bohr effect (16). The acid bohr effect may be the result of interactions between many groups which have not been identified yet. It seems likely that the alkaline and acid parts of the bohr effect are independent (16).

The α-amino parts of the N-terminal valines of both the α- and the β-chains participate in the oxygen-linked binding of CO_2. This oxygen-linked CO_2 binding is the cause of the shift in p_{50} occurring when pCO_2 changes at a fixed pH, *i.e.* the CO_2 bohr effect.

For the sake of simplicity, the differences between the proton and CO_2 binding properties of the α- and β-chains of the hemoglobin molecule were neglected, as were the interactions between them. The monomers were assumed to behave identically, in line with the original assumption of Wyman (30). The affinities to protons and CO_2 as used in the model can be considered to represent mean values of the affinities of the proton and CO_2 binding groups to the α- and β-chains in the presence of a normal 2,3-DPG concentration. To account for the fact that in the N-terminal valines of the α-chains CO_2 binding partly interferes with proton binding, each monomer was assumed to have two alkaline bohr groups instead of one. To one of these groups oxygen-linked CO_2 and proton binding was ascribed, the other group exhibited only oxygen-linked proton binding.

As already mentioned, the classical bohr effect concerns whole blood and is due to the change in pCO_2 and the concomitant change in pH. To separate these two influences one of the two variables, pCO_2 or pH, was varied and the other was kept constant. Thus, the H^+ bohr factor of blood with respect to p_{50} is measured as the change in log (p_{50}) divided by the change in blood (plasma) pH at constant pCO_2. The CO_2 bohr factor of blood is accordingly determined at constant blood (plasma) pH. The relation between the classical bohr factor and the H^+ and CO_2 bohr factors is

$$(38) \quad \frac{d \log (p_{50})}{d \log (pCO_2)} = \left. \frac{\partial \log (p_{50})}{\partial \log (pCO_2)} \right|_{pH_p} + \left. \frac{\partial \log (p_{50})}{\partial pH_p} \right|_{pCO_2} \cdot \frac{dpH_p}{d \log (pCO_2)}$$

Thus in contrast with the H^+ and CO_2 bohr factors of blood, the classical bohr factor also depends on the CO_2 buffer capacity of the blood. Direct calculation of the classical bohr factor by simulating titration of blood with CO_2 gave -0.33 at $pH_p = 7.4$, $pCO_2 = 5.33$ kPa, and $T = 310$ K. The classical bohr factor is often expressed with respect to the uncontrolled change in blood pH concomitant with the change in pCO_2. The reason for this is that the measurement of pH is easier, more accurate, and more reliable than that of pCO_2. The value calculated for this quantity was -0.51 at $pH_p = 7.4$, $pCO_2 = 5.33$ kPa, and $T = 310$ K. This agrees with comparable experimental
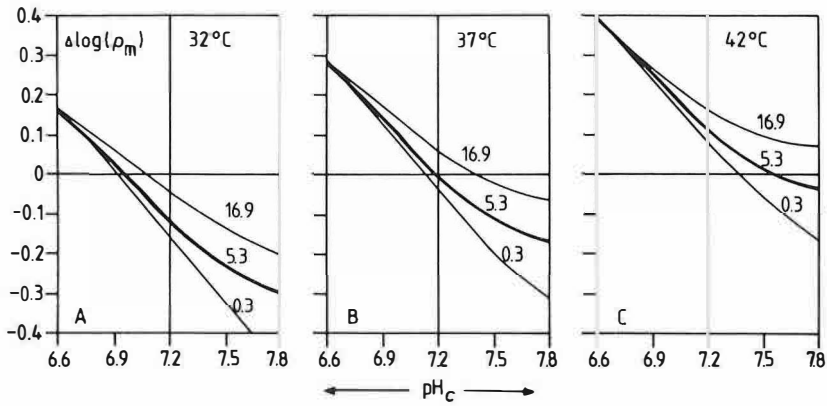
Fig. 1. Change in log (p_m) of a hemoglobin solution *versus* pH in the solution at three values of pCO_2 (kPa). Panel A: 32 °C (305 K); panel B: 37 °C (310 K); panel C: 42 °C (315 K).

values given in the literature: -0.54 ± 0.06 (12), -0.53 (9), and -0.51 (21). Hilpert *et al.* (12) also determined the ratio between Δ log (p_{50}) and the change in red cell pH. They have found this to be -0.66 ± 0.08. The value of this quantity as calculated with our model was -0.65.

Some properties of hemoglobin in a solution representing erythrocyte content as calculated with the equations derived are given in figs. 1 to 5. Figs. 1 to 3 show the change in log (p_m) dependent on the pH, pCO_2, and temperature of the hemoglobin solution. Figs. 4 and 5 give the H^+ and the CO_2 bohr factors, respectively. Because these curves concern a hemoglobin solution they also represent the change in H^+ and CO_2 bound per mol hemoglobin monomer, *i.e.* the haldane factors for hemoglobin. The absolute value of the H^+ bohr factor at low pCO_2 is 0.57. As already mentioned, this value was reached by
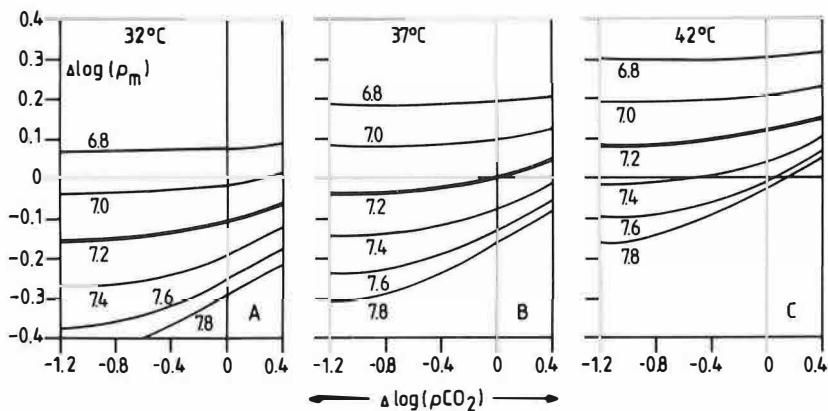
Fig. 2. Change in log (p_m) of a hemoglobin solution *versus* pCO_2 at six values of pH in the solution. Panel A: 32 °C (305 K); panel B: 37 °C (310 K); panel C: 42 °C (315 K). $pCO_2 = 5.3$ kPa at Δ log (pCO_2) = 0.

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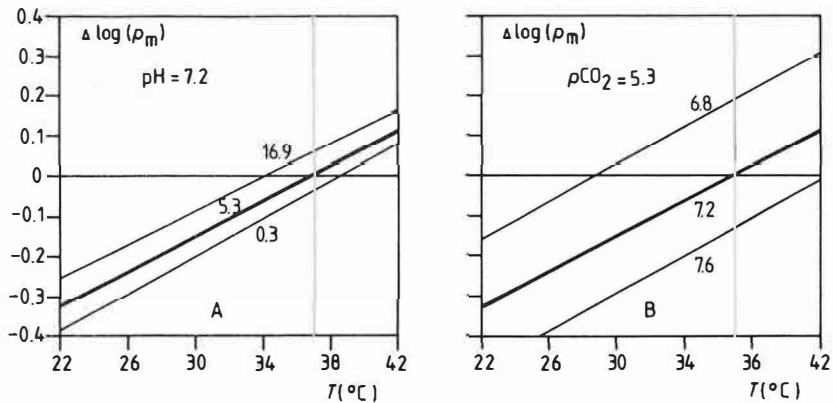
Fig. 3. Change in log (p_m) of a hemoglobin solution *versus* temperature. Panel A: pH = 7.2 at three values of pCO_2 (kPa); panel B: $pCO_2 = 5.3$ kPa at three values of pH.

a suitable choice of pK_{zo} and pK_{zd}. The H^+ haldane factor thus calculated is in agreement with that experimentally determined in erythrolysate by Siggaard-Andersen [27] and is slightly lower than found by Horvath *et al.* [14] for the H^+ bohr factor for a concentrated hemoglobin solution (0.64). On the whole, fig. 4A is in good agreement with the experimental results concerning the influence of CO_2 on the proton haldane factor as given by Siggaard-Andersen [27].

Figs. 6, 7, 8, and 9 show the relations between p_m and plasma values for blood in a similar way as figs. 1, 2, 3, and 4 show these for the hemoglobin solution. The difference with the latter is that here plasma pH is referred to, instead of the pH of the solution which contains the hemoglobin. The curves for blood are related to those of the hemoglobin solution through a transfor-
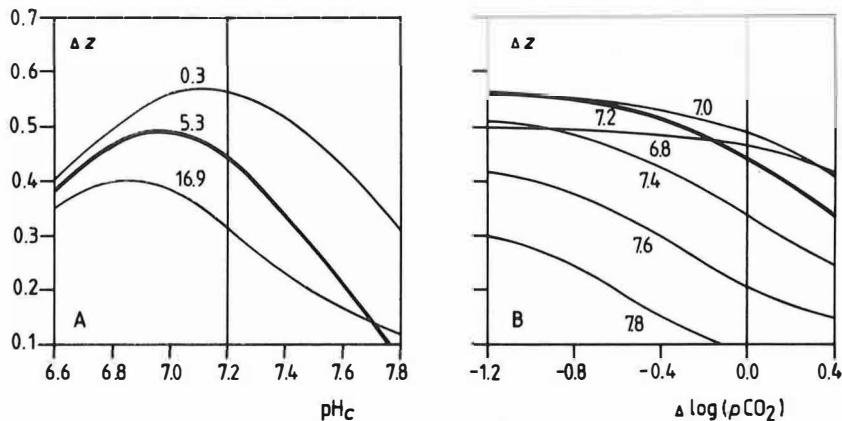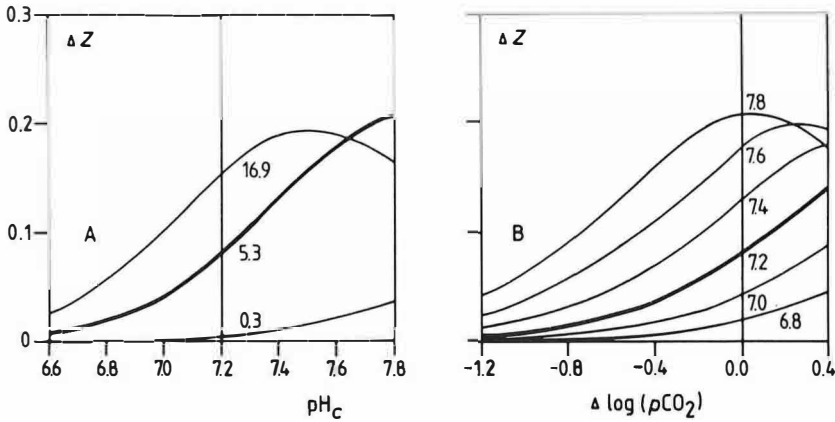

Fig. 4. Proton haldane factor (Δz) of hemoglobin in solution at 37 °C (310 K). Panel A as a function of pH at three values of pCO_2 (kPa); panel B as a function of log (pCO_2) at six values of the pH. The proton bohr factor is equal to the proton haldane factor (equation 3). $pCO_2 = 5.3$ kPa at $\Delta \log (pCO_2) = 0$.

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Fig. 5. CO_2 haldane factor (ΔZ) of hemoglobin in solution at 37 °C (310 K). Panel A as a function of pH at three values of pCO_2 (kPa); panel B as a function of log (pCO_2) at six values of the pH. The CO_2 bohr factor is equal to the CO_2 haldane factor (equation 4). $pCO_2 = 5.3$ kPa at Δ log (pCO_2) = 0.

mation of the pH. The plasma pH differs from the pH of the intracellular hemoglobin solution by an amount equal to the 10 logarithm of the donnan ratio between the plasma and the intracellular solution. The donnan ratio depends on pH and pCO_2 and was calculated with the set of equations presented earlier (33).

In blood the bohr and haldane factors are not equal. This is shown in figs. 9 and 10. The curves give the H^+ and CO_2 bohr and haldane factors as calculated for human blood. Fig. 9A shows that the difference between the H^+ bohr and the H^+ haldane factors in blood increases with increasing pCO_2. In the presence of CO_2 the H^+ haldane effect is smaller than the H^+ bohr effect. In contrast with this, the CO_2 haldane effect in blood is larger than the CO_2 bohr effect (fig. 10).
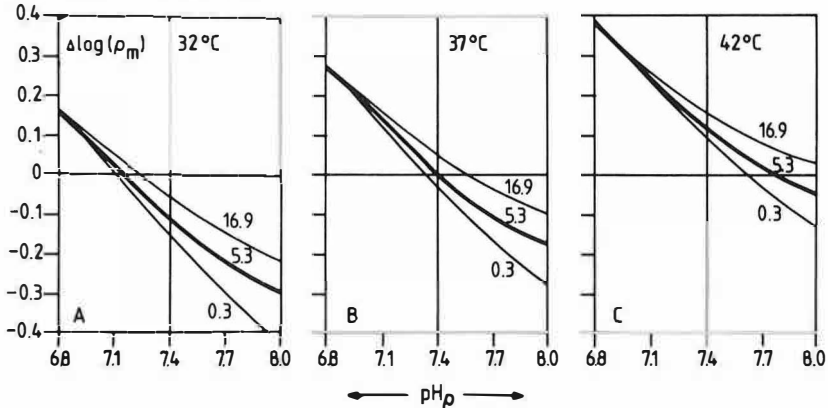

Fig. 6. Change in log (p_m) of blood *versus* plasma pH at three values of pCO_2 (kPa). Panel A: 32 °C (305 K); panel B: 37 °C (310 K); panel C: at 42 °C (315 K).
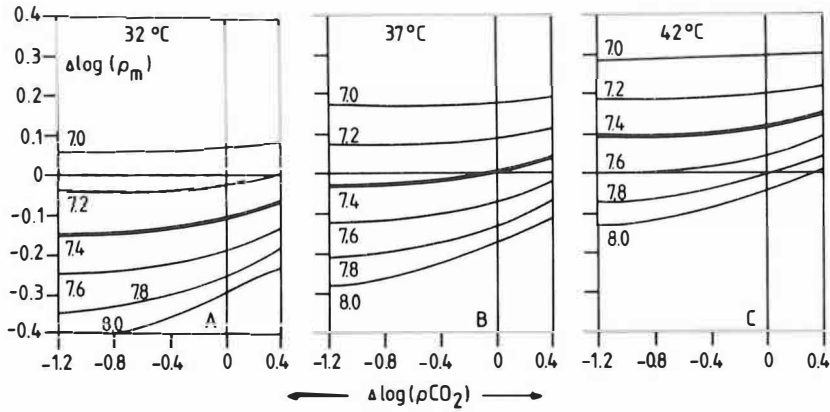
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Fig. 7. Change in log (p_m) of blood *versus* log (pCO_2) at six values of plasma pH. Panel A: 32 °C (305 K); panel B: 37 °C (310 K); panel C: 42 °C (315 K). $pCO_2 = 5.3$ kPa at Δ log (pCO_2) = 0.

The difference between the bohr and haldane factors in blood results from the fact that the buffering of H^+ not only takes place in the hemoglobin solution inside the erythrocytes but also in plasma. Hemoglobin, however, which is intracellular, only "sees" intracellular pH. This pH is related to plasma pH through the osmotic and donnan equilibrium conditions. The consequence of this is that although plasma pH is kept fixed while changing sO_2, intracellular pH is not. Changes in intracellular pH give rise to changes in the amounts of both protons and CO_2 bound to hemoglobin. For blood therefore, it is not possible to separate the bohr effect of protons from that of CO_2 on the guidance of plasma values. So, when measured in whole blood, the H^+ bohr factor includes a CO_2 bohr effect and the CO_2 bohr factor includes a proton effect. Furthermore, as the buffer properties of hemoglobin depend
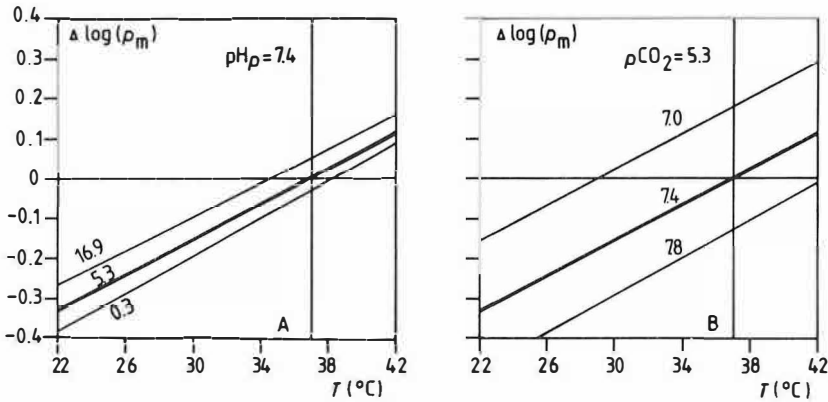

Fig. 8. Change in log (p_m) of blood *versus* temperature. Panel A: plasma pH 7.4 at three values of pCO_2 (kPa); panel B: $pCO_2 = 5.3$ kPa and three values of pH.
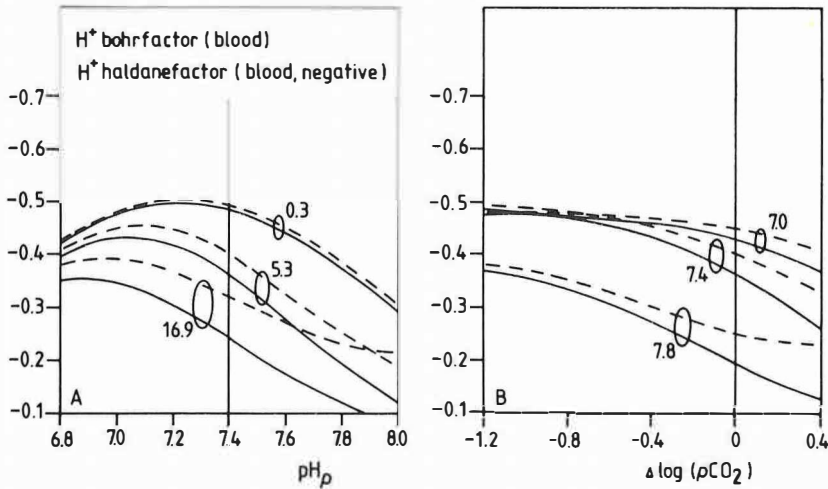
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Fig. 9. Proton bohr factor (broken lines) and proton haldane factor (drawn lines) in blood at 37 °C (310 K). Panel A as a function of plasma pH at three values of pCO_2 (kPa); panel B as a function of log (pCO_2) at three values of plasma pH. The haldane factor is smaller than the bohr factor, the difference getting larger at increasing pCO_2. $pCO_2 = 5.3$ kPa at Δ log (pCO_2) = 0.

on oxygen saturation, the bohr factors in whole blood also depend on oxygen saturation, because at different degrees of saturation equal changes in plasma pH do not give rise to changes of equal magnitude in red cell pH. This phenomenon is reflected in the fact that in whole blood the unconstrained change in plasma pH following upon a change in pCO_2 is oxygen saturation
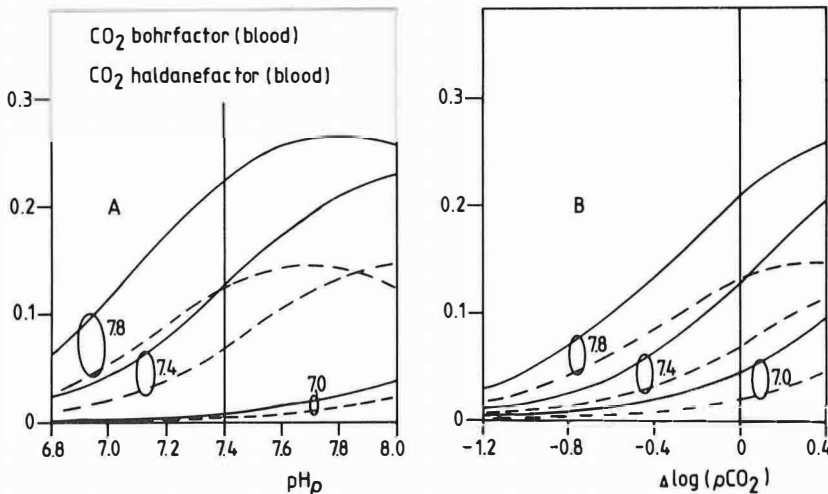

Fig. 10. CO_2 bohr factor (broken lines) and CO_2 haldane factor (continuous lines) in blood at 37 °C (310 K). Panel A as a function of plasma pH at three values of pCO_2 (kPa); panel B as a function of log (pCO_2) at three values of plasma pH. The CO_2 haldane factor is larger than the CO_2 bohr factor. $pCO_2 = 5.3$ kPa at Δ log (pCO_2) = 0.
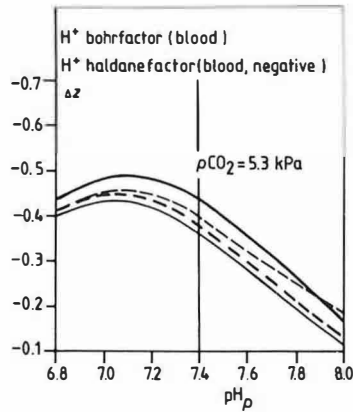
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Fig. 11. Proton bohr factor of blood (broken line), proton haldane factor of blood (continuous line), and bohr and haldane factors of a hemoglobin solution representing red cell content at $sO_2 = 0.5$ (continuous thick line), and the change in z of hemoglobin between deoxygenated and oxygenated blood (broken thick line) *versus* plasma pH at $pCO_2 = 5.3$ kPa and $T = 310$ K.

dependent. From fig. 9A it can be read that at $pCO_2 = 5.3$ kPa and $pH_p = 7.4$ the H^+ haldane factor calculated for blood is -0.36. This value is in good agreement with the value of -0.35 measured by Arczynska and Held (3). The bohr factor calculated under these conditions was -0.40.

A comparison of bohr factors in whole blood from the literature with those calculated here is given in table 3. As far as possible the mutual influences of pH and pCO_2 on the bohr factors were calculated. These are also given in table 3. There is a reasonable agreement between the experimental and the calculated values. Figs. 11 to 16 give the H^+ and CO_2 bohr and haldane factors as calculated for whole blood. In figs. 12 and 14 the whole blood H^+ and CO_2 haldane factors are given together with the contributions made by hemoglobin.
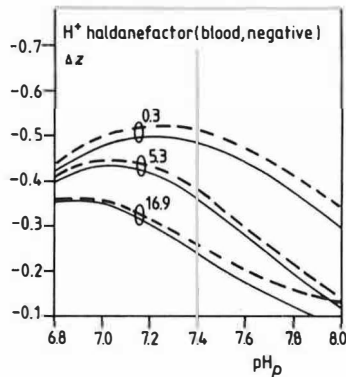

Fig. 12. Proton haldane factor of blood (continuous lines) and the change in z of hemoglobin between oxygenated and deoxygenated blood (broken thick lines) *versus* plasma pH at three values of pCO_2 (kPa).

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Table 3. H^+ and CO_2 bohr effects and their mutual dependence as measured in and calculated for human blood at 37 °C with pCO_2 or pH as indicated.

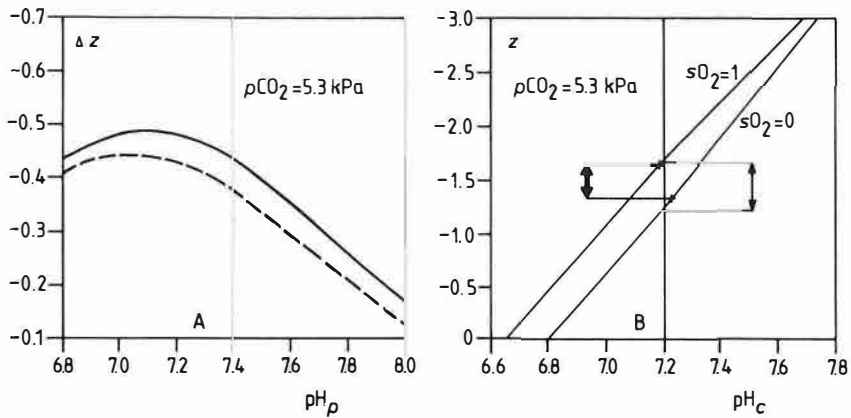| Source	$\dfrac{\Delta \log (p_{50})}{\Delta pH_p}$	$\dfrac{\Delta}{\Delta \log (pCO_2)}\left(\dfrac{\Delta \log (p_{50})}{\Delta pH_p}\right)$	$\dfrac{\Delta \log (p_{50})}{\Delta \log (pCO_2)}$	$\dfrac{\Delta}{\Delta pH_p}\left(\dfrac{\Delta \log (p_{50})}{\Delta \log (pCO_2)}\right)$
Arturson et al. (4)	−0.39 (5.3 kPa)	0.149	0.06 (7.4)	0.10
Garby et al. (9)	−0.38 (4.5 kPa)	0.19	0.10 (7.4)	0.20
			0.06 (7.2)	
Hlastala and Woodson (13)	−0.399 (5.6 kPa)		0.0514 (7.4)	
Horvath et al. (14)	−0.395 (5.6 kPa)			
Winslow et al. (29)	−0.36 (5.3 kPa)	0.123	0.038 (7.36)	0.14
	−0.33 (9.3 kPa)		0.045 (7.51)	
Zijlstra et al. (32)	−0.396 (5.3 kPa)	0.038	0.068 (7.4)	0.038
Calculated (this paper)	−0.40 (5.3 kPa)	0.18	0.068 (7.4)	0.18

Fig. 13. Explanation of the fact that the amount of protons released per hemoglobin monomer (Δz) is smaller when blood is oxygenated at constant pH and pCO_2 than when the same is done with a hemoglobin solution. Panel A: change in z of hemoglobin in solution (continuous thick line) and in blood as a function of plasma pH (broken thick line). Panel B: z as a function of intracellular pH (pH_c) for oxygenated and deoxygenated hemoglobin. The thin arrow indicates the change in z at constant pH in the solution, the thick arrow at constant plasma pH. In the latter case the pH inside the red cells changes in such a way that Δz is smaller than when no such a change in pH occurs.

In fig. 11 besides the H^+ bohr and haldane curves, two other curves are given. The uppermost curve gives the bohr factor in a hemoglobin solution at the intracellular pH and pCO_2 that result from the plasma pH at that pCO_2 with $sO_2 = 0.5$. Because it concerns a solution, the bohr factor is equal to the haldane factor. Below the broken thin line of the blood H^+ bohr factor and above the continuous thin line of the H^+ haldane factor of blood, the broken thick line represents the change in z of hemoglobin between $sO_2 = 0$ and $sO_2 = 1$. In fig. 12 the change in z is compared with the H^+ haldane effect of
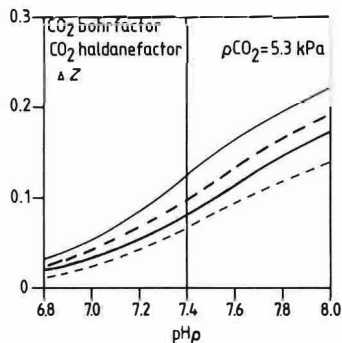

Fig. 14. CO_2 bohr factor of blood (broken thin line), CO_2 haldane factor of blood (continuous thin line), CO_2 bohr and haldane factors of a hemoglobin solution representing red cell content at $sO_2 = 0.5$ (continuous thick line), and the change in Z of hemoglobin between deoxygenated and oxygenated blood (broken thick line) *versus* plasma pH at $pCO_2 = 5.3$ kPa and $T = 310$ K.
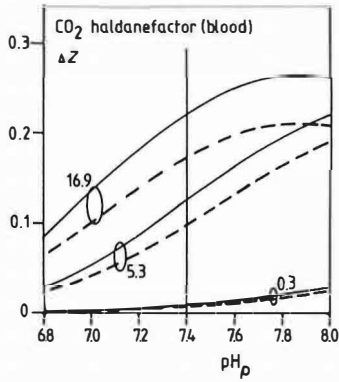
Fig. 15. CO_2 haldane factor of blood (drawn lines) and the change in Z of hemoglobin between oxygenated and deoxygenated blood (broken thick lines) *versus* plasma pH at three values of pCO_2 (kPa).

blood for three different values of pCO_2. The haldane factor is smaller than the change in the amount of protons bound to hemoglobin. This is due to the action of other buffers in blood, notably plasma protein, ATP and 2,3-DPG. Fig. 13A presents the curve of the bohr factor of hemoglobin in solution and that of Δz between $sO_2 = 0$ and $sO_2 = 1$. In a solution the bohr factor equals Δz, but in blood it does not because then intracellular pH is not constant. This is illustrated in fig. 11B. In figs. 14, 15, and 16 similar pictures are given for the effects of CO_2. These, however, are larger in blood than in a hemoglobin
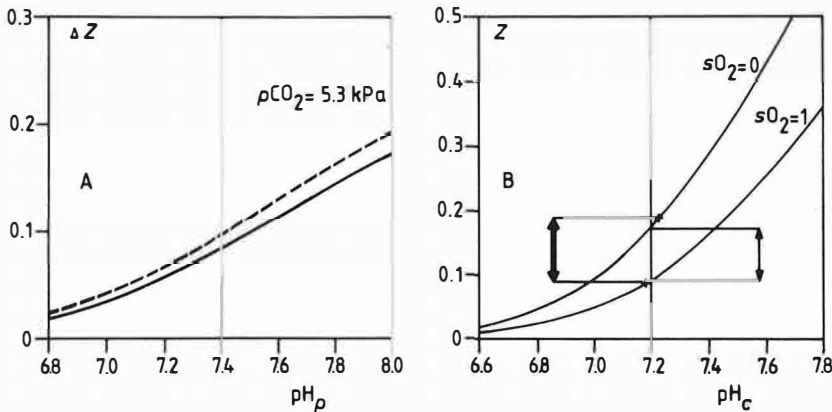

Fig. 16. Explanation of the fact that the amount of CO_2 released per hemoglobin monomer (ΔZ) is larger when blood is oxygenated at constant pH and pCO_2 than when the same is done with a hemoglobin solution. Panel A: change in Z as a function of plasma pH in a hemoglobin solution (continuous thick line) and in blood (broken thick line). Panel B: Z as a function of intracellular pH (pH_c) for oxygenated and deoxygenated blood. The thin arrow indicates the change in Z at constant pH in the solution, the thick arrow at constant plasma pH. In the latter case the pH inside the red cells decreases which leads to an increase in ΔZ.

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solution. The mechanism which makes the H^+ effects in blood smaller than in a solution, works in the case of CO_2 just the other way round. The buffering by plasma proteins, ATP, and 2,3-DPG makes that more CO_2 can be accomodated in blood than in hemoglobin solution. The shift in pH_c also acts in favour of CO_2 transport, as is illustrated in fig. 16B.

The titration curve of hemoglobin depends on sO_2. When sO_2 changes, the donnan ratio between intra-erythrocytic solution and plasma changes as well. Thus, although plasma pH, pCO_2, and temperature are kept constant, intra-erythrocytic pH changes with sO_2. This also causes p_{50} to change. Under these conditions, points of the OEC measured in whole blood do not lie on one and the same true hemoglobin-OEC because, as far as pH is concerned, the intra-erythrocytic environment for hemoglobin is not constant. An OEC measured in blood is therefore not a "true" OEC, for which all intracellular quantities except pO_2 remain constant.

With the assumption that the OEC calculated with equation 36 is correct for blood, the OEC for constant intra-erythrocytic values was calculated. $sO_2 = 0.5$ was taken as the reference value. The difference between the OEC for blood and the "true" OEC of hemoglobin is small. There are two extremes in the difference, one between $sO_2 = 0$ and $sO_2 = 0.5$, in which case it was about -0.02 kPa at about $sO_2 = 0.18$, and a positive, infinitely large one at $sO_2 = 1$ and pO_2 infinite. The lower value was calculated with $pH_p = 7.4$, $pCO_2 = 5.3$ kPa, and $T = 310$ K. On the whole, the OEC measured in blood is less curved than that of the intra-erythrocytic hemoglobin solution, but for all practical purposes the difference is negligibly small.

The buffer capacity of blood depends on the oxygen saturation of the hemoglobin. Because of this the position of the CO_2 bufferline of blood is sO_2 dependent. In the HCO_3^-/pH_p diagram CO_2 bufferlines of blood with different sO_2 are almost parallel to each other. The bufferline with $sO_2 = 0$ is the uppermost bufferline, that with $sO_2 = 1$ the lowest one. However, at constant pO_2, the sO_2 depends on pH and pCO_2 as well. Blood exposed to a certain pO_2
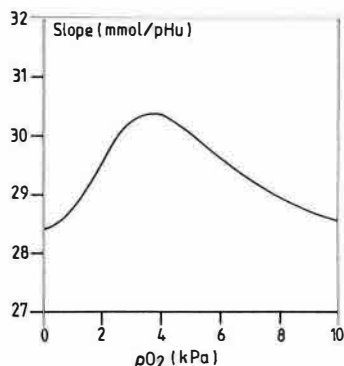

Fig. 17. Slope of CO_2 bufferline of blood at constant pO_2 *versus* the pO_2 at which the bufferline is calculated.

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in the range 0–10 kPa that is titrated with CO_2 to measure the CO_2 bufferline does not have a constant sO_2. It has a lower sO_2 in the lower pH range $i.e.$ at a high pCO_2, and a higher sO_2 in the higher pH range, $i.e.$ at a low pCO_2. Such a CO_2 bufferline thus crosses the CO_2 bufferlines with constant sO_2. The effect is that at certain pO_2 in the range 0–10 kPa, the slope of the bufferline is dependent on pO_2. The slope as calculated for these conditions is given in fig. 17.

The model presented does not represent the most recent state of affairs concerning research on oxygen binding by hemoglobin. In the MWC (Monot, Wyman, Changeux) model of hemoglobin (19), the molecule is thought to switch over between a restricted number of states. The most important switch is that between two quaternary structures, the R state, with a high oxygen affinity, and the T state, with a low affinity. At increasing pO_2, the ratio between the number of R state molecules and the number of T state molecules increases, because the stability of the R state increases on oxygen binding. Thus, the average affinity to oxygen increases.

The model of hemoglobin used here, however, perhaps does not differ so much from the two state MWC model as it may seem. The hemoglobin molecule was assumed to be in one of the two states: oxygenated or deoxygenated, each state characterized by its affinities to H^+ and CO_2. Instead of the oxygenated and the deoxygenated states, one could substitute the R and T state. In terms of this model, the oxygen affinity modifiers H^+ and CO_2 can be considered to influence the average oxygen affinity expressed as p_m by changing the ratio between the amounts of molecules in the R and the T state. They could induce this change by influencing either the affinities of the monomers for oxygen or the free energies in the two states or both. In this way, the description of the properties of hemoglobin presented can be interpreted as conforming to a two state model. For the description of the macroscopic behaviour of hemoglobin under equilibrium conditions, these differences in interpretation are not very relevant.

In the model presented, a number of assumptions concerning the properties of hemoglobin were discussed. Apart from the simplifications applied, the main disadvantage of the model presented is that 2,3-DPG only acts as a buffer substance that is osmotically active but not as a modifier of oxygen affinity. This disadvantage was met in so far that the values of pK's used lead to a description of the properties of hemoglobin as these are in the presence of normal amounts of 2,3-DPG. Moreover, the assumption that the kations other than H^+ cannot leave their compartment may not always lead to completely realistic results. Nevertheless, the model provides useful insight into the interactions between pH, pCO_2 and the resulting oxygen affinity of blood.

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CHAPTER IV

INFLUENCE OF TEMPERATURE ON CO_2 AND O_2
EQUILIBRIA IN HUMAN BLOOD

INFLUENCE OF TEMPERATURE ON CO_2 AND O_2 EQUILIBRIA
IN HUMAN BLOOD

ABSTRACT

In order to study how various physico-chemical quantities in blood interact, an equilibrium model of blood has been formulated. The set of equations with appropriate values for a number of quantities, together comprising the model, have been implemented in a computer program. This program makes it possible to calculate, among other things, the acid base equilibria of blood *in vitro* in closed system or in isocapnic conditions at temperatures other than 37 °C. Results of these calculations are presented in the form of diagrams.

The results show that when the temperature decreases and carbon dioxide tension is kept constant, pH of plasma remains almost constant at the starting value of 7.41 at 37 °C. Under conditions where the total CO_2 content of the blood remains constant, the pH of plasma rises with decreasing temperature. This rise is parallel with the increase in the pH of pure water (pN) with decreasing temperature, the pH of plasma being about 0.6 unit above pN. The slope of the plasma pH *vs* temperature curve was calculated to be -0.0169 /K when $cHb = 150$ g/L, which is slightly higher than the experimental values.

When the total CO_2 content is kept constant, the decrease in pCO_2 with decreasing temperature is fairly approximated by a linear relation between $\log(pCO_2)$ and T, with a slope of 0.023 /K. This slope is in fair agreement with experimental values. It follows from this relationship that after anaerobic cooling to 22 °C, the pCO_2 of the blood that is normocapnic at 37 °C (5.3 kPa) is about 2 kPa.

The results also show that under the condition of a closed system the donnan ratio between plasma and erythrocytes hardly changes with changes in temperature, while when pCO_2 in the blood is kept constant during cooling, the donnan ratio increases with decreasing temperature. The same applies to the hematocrit. These results are in agreement with experimental findings from the literature. Furthermore, it was calculated that when total CO_2 content is kept constant, the fall in pCO_2 with decreasing temperature is almost independent of the hematocrit. This finding supports the theory by Severinghaus (1959) and by Rahn, Reeves and Howell (1975) that in a patient under induced hypothermia the total CO_2 content of arterial blood should be kept constant by way of a suitable alveolar ventilation.

INTRODUCTION

The influence of temperature on the gas transport by and the acid-base balance of the blood regained interest with the theories put forward by Rahn, Reeves, and Howell (32). They put forward a rather convincing hypothesis about which blood gas values are "normal" for homeotherms at temperatures other than the normal body temperature. Their conclusion was based on observations made in ectothermic vertebrates. These animals regulate their pH by means of the arterial pCO_2 in such a way that, at any temperature in the

physiological range, the difference between plasma pH and the neutral point of water, pN, is maintained at a constant value of about 0.6. Some years ago, we formulated an equilibrium model of human blood in order to study interactions between various physico-chemical quantities in blood (52). The set of equations with the appropriate values for a number of constants have been implemented in a computer program. This computer program offers the possibility to calculate the acid-base equilibria of human blood *in vitro* under a variety of conditions including temperatures other than 37 °C (310 K). Two types of equilibrium conditions will be compared here: blood cooled down or warmed up at a constant pCO_2 and blood cooled down or warmed up with a constant total CO_2 content (tCO_2). In both conditions a number of quantities were calculated for temperatures between 17 and 42 °C (290-315 K) with intervals of 5 °C. The two conditions may be seen as representing two policies advocated for the ventilation of patients under hypothermia. The results show that in blood in closed system conditions, the donnan ratio and the hematocrit are nearly independent of temperature, which is in contrast with the results calculated for constant pCO_2. Moreover, under these conditions, the difference between plasma pH and pN is also almost constant at 0.6 in the range of temperature from 17 to 42 °C. This supports the extension of the original hypothesis by Rahn (31) put forward by Rahn, Reeves and Howell (32) which states that the "normal" blood gas values at temperatures other than 37 °C are those at which the arterial CO_2 content is equal to that at 37 °C.

The study of the deterioration of physiological functions in homeotherms at low body temperatures goes back at least as far as 1862. In that year Walther (50) published his "Beitrage zur Lehre von der thierischen Wärme". He found that rabbits at a body temperature of 20 °C did not shiver and would have died if left in a cool place, but that they could be revived by means of artificial ventilation, with their body temperature slowly rising, even if the air temperature was only 10 °C. With these experiments Walther contributed to the discussion on the matter of the "animal heat". He claimed that this was the first proof that the generation of animal heat depends on the entrance of air into the lungs.

The systematic study of the influence of body temperature on the physiological functions of homeotherms started only eighty years later with the study by Dill and Forbes (18) on the respiratory and metabolic effects of hypothermia. The impetus to this study was the need for insight into such matters as adaptation to and protection against cold and the nature of the breakdown resulting from prolonged exposure to cold. This need was born from experiences of troops in Finland, Norway and Greece and of armed forces in the arctic and at high altitude. During World War II it became clear from the ob-

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servation of cases of drowning in cold sea-water that the most important threat to life for those who kept afloat was the cooling down, leading to certain death (23). Answers to questions raised about hypothermia called for knowledge of metabolic, respiratory and cardiovascular response to cold. In 1940 Smith and Fay (46), in an attempt to treat cancer, for the first time induced in man body temperatures as low as 24 °C. As an outgrowth of this, an experimental hypothermic treatment of schizophrenic patients was instituted. In retrospect, it is clear that the application of the – at the time – new technique was pure magic. It makes one wonder how years hence today's modern medicine will be looked upon. The observations made by Dill and Forbes (18) concern these schizophrenic patients. The patients were cooled down externally as far as a lowering of skin temperature by 20 °C. They were lightly anaesthetized; after 1 to 4 hours cold itself provided the only anaesthesia. The patients were breathing spontaneously and besides a glucose solution given by stomach tube, no measures were taken to control the state of the patient.

Dill and Forbes found that the ventilation had increased out of proportion to the increased oxygen consumption. Arterial blood samples were interpreted as showing the patients to be in a state of metabolic acidosis caused by the shivering and the struggling of the patients and partially compensated for by hyperventilation. This interpretation was more or less a guess, because to judge the acid-base status of blood at temperatures other than 37 °C one will first have to decide what state should be considered "normal" and thus be used as the reference. Until now, no consensus of opinion has been reached about this.

The question of which blood gas values and which acid-base status should be taken as optimal at lower body temperatures became even more important when controlled hypothermia was introduced into regular medical practice. The clinical application of hypothermia was the sequel to experimental cardiotomy during inflow occlusion on dogs under deep hypothermia by Bigelow *et al.* in 1950 (9). Similar experiments were done by Boerema *et al.* (11) in 1951. In 1953 Swan *et al.* (48) and Lewis and Taufic (25) reported successful open heart surgery with circulatory occlusion under hypothermia. Since then, cardiac surgery under deep hypothermia has developed into a technique with its own specific indications. When the optimal blood gas values and acid-base status in hypothermia are known, a part of the control effort can be aimed at maintaining or approaching this optimal condition in the patient as closely as possible.

In the absence of a clear-cut idea about which condition is the normal, or strickly speaking the optimal, blood gas and acid-base condition, many policies were advocated for the regulation of ventilation and plasma pH under hypothermia. These were often at variance with each other, indicating the lack of a

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generally accepted theory. Considering the application, one can understand that the behaviour of the heart shortly before and after the arrest during hypothermia was taken as an important indicator for judging the quality of the policy used. Two properties were considered especially important: irritability and cardiac performance. The first as indicating whether the heart will be able to regain a stable and normal rhythm, the second as a necessity to sustain life. The findings concerning these properties did anything but lead to a conclusive answer as to which pCO_2 and pH should be aimed at in deep hypothermia.

For the clinical application of hypothermia, the question of which oxygen tension (pO_2) should be considered normal at a lower body temperature is probably less important. As long as the oxygen saturation (sO_2) of the arterial blood is almost complete, the oxygen supply of the tissues only depends on their perfusion. This again stresses the importance of acid-base conditions that allow optimal functioning of the heart, which, in turn, is necessary to protect so vulnerable an organ as the brain from a shortage of oxygen. As for the brain, a correct choice of the pCO_2 is important in another way too. The vascular resistance of the brain, and therefore its perfusion and thus the oxygen supply, strongly depends on the pCO_2. Regarding the control of the patient, the maintenance of complete oxygen saturation practically means that alveolar pO_2 has to be kept sufficiently high.

It is important to know whether under hypothermia the pO_2 at capillary oxygen saturations between 0.7 and 1 is sufficiently high to guarantee an adequate supply of oxygen to even the most vulnerable tissue cells. Some studies (10) indicate that this is indeed the case, so that as far as the oxygen supply is concerned, full saturation of arterial blood should be aimed at in patients with controlled ventilation. If necessary, it is possible, although difficult, to raise the pO_2 in the tissues above that concomitant with normal values of the oxygen saturation of hemoglobin, $i.e.$ sO_2 between 0.7 and 0.95. In that case $sO_2 = 1$ and the oxygen supply has to come from dissolved O_2 so that the arterial pO_2 must be rather high. A high pO_2 in the tissues is hard to control because the differential storing capacity of blood is low at full saturation. During prolonged application of a high pO_2 the possibility of oxygen toxicity should be considered.

In patients breathing spontaneously, alveolar ventilation will diminish under the anaesthetic action of hypothermia, and therefore alveolar pO_2 tends to diminish as well. In accidental hypothermia this may lead to an insufficient supply of oxygen. To be able to judge at which pO_2 one should intervene with assisted ventilation, the critical value of the arterial pO_2 should be known. Assuming that a normal arterial oxygen saturation complies with a pO_2 that is sufficient to provide the necessary oxygen to the tissues, this pO_2 can be

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taken from the oxygen equilibrium curve (OEC) at the prevailing temperature, provided the corresponding optimal pH and pCO_2 are known. Thus, we are again confronted with the question of what pH and pCO_2 values are to be considered optimal at deviating body temperatures.

As early as 1959, Severinghaus (45), in considering that either pH or pCO_2 might arbitrarily be held constant, reasoned that the rapid change in both pH and pCO_2 in anaerobically cooled blood detracts from the rationality of fixing one of them in controlling the acid-base status of arterial blood in artificially ventilated patients under hypothermia. He proposed that ventilation in hypothermic patients should be of such a rate that carbon dioxide elimination equals its rate of metabolic production. This rate will diminish as cooling progresses. Severinghaus concluded from this that the total CO_2 content of the blood should remain constant and that, by consequence, the pH of the arterial blood *in vivo* should rise at the same rate as in blood anaerobically cooled *in vitro*, i.e. by the rosenthal factor of 0.0147 /K (44). Furthermore, arterial pCO_2 should fall at the same rate as the pCO_2 of normal arterial blood cooled anaerobically *in vitro*, reaching about 3 kPa (22.5 mmHg) at 25 °C (297 K). This pCO_2 should be used to define the ventilation. The arterial pCO_2 should therefore be equal to the pCO_2 of blood of normal composition at 37 °C which has been anaerobically cooled to the prevailing temperature. Metabolic acidosis would present itself as a fall in tCO_2 and a less than expected rise in pH. Severinghaus stressed that this ventilation is not necessarily the best ventilation to ensure optimal protection of the circulation in hypothermia. He considered this, therefore, to be a separate problem which should be solved experimentally.

Severinghaus found that the desired decrease in arterial pCO_2 could be reached by the use of approximately the same tidal volume and rate of ventilation as needed to maintain a pCO_2 of 5.3 kPa (40 mmHg) at normal body temperature. Surprisingly, this paper by Severinghaus has not been quoted by Rahn or his coworkers in their papers concerning this matter.

The consequences of three control policies for the acid-base status of arterial blood under hypothermia were calculated by Albers in 1962 (3). The policies concerned were: constant ventilation, constant CO_2 content, and constant pCO_2. In contrast with the findings of Severinghaus, Albers' calculations predict a fall in tCO_2 at constant ventilation. To keep tCO_2 constant, ventilation should be about halved at 20 °C; to keep pCO_2 constant the ventilation should even have to be lowered to about one fourth.

To be able to calculate the ventilation, Albers assumed that the metabolic production of CO_2 depends exponentially on temperature. A fall in temperature of l0 °C would diminish CO_2 production by a factor of 2.5. At constant tCO_2 the arterial pH was calculated to increase linearly with the diminishing tem-

perature to about 7.65 at 20 °C. At constant ventilation the pH rose even higher with falling temperature. A constant pCO_2 gave an almost constant pH, nearly independent of temperature. This result is in agreement with the experimental findings of Brewin *et al.* (13). Besides these mutual relations between tCO_2, pCO_2, and pH, Albers calculated the consequences of the three policies on the ratio of (OH^-) to (H^+). This ratio was the least influenced by changes in temperature when tCO_2 was held constant. For the importance of this quantity Albers referred to Winterstein (51), who reasoned that at a deviating body temperature the $(OH^-)/(H^+)$ ratio should be taken as the measure of the acid-base status instead of pH. The ratio $(OH^-)/(H^+)$ is equivalent to pH-pN as an indicator of the deviation from neutrality, as can be easily shown [1]). The importance of the properties of water in relation to changes in temperature was clear to Winterstein, who referred to Henderson (21) for this matter. He even quoted Henderson to show that the latter had been well aware that the change in pH, or rather its complement, the "alkalinity", with temperature should be considered in connection with the concomitant change in the dissociation constant of water K_w. This reasoning was used by Winterstein as an argument to reject pH as a measure of the acid-base status of the blood when changes in temperature take place in favour of the $(OH^-)/(H^+)$ ratio.

Although Austin and Cullen (5) appear to have been the first to propose that evaluation of the acid-base balance at different temperatures requires a consideration of the $(OH^-)/(H^+)$ ratio, they were not the first who used this ratio. In 1919 Moore (28) introduced the $(OH^-)/(H^+)$ ratio in a paper in which he discussed the extreme sensitivity of living systems to small changes in pH. At the time it was supposed that proteins reacted with both H^+ and OH^- ions. From this, Moore deduced that not the concentration of one of these ions was important but their ratio. He used this ratio as a general measure of the deviation from neutrality, not explicitly for cases in which changes in tem-

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[1]) Dissociation of water, equilibrium condition: $(OH^-) \cdot (H^+) = K_w$.

Taking the [10]log of this and changing signs gives:

$$pOH + pH = pK_w = 2\ pN \text{ and } pH - pN = pOH - pN.$$

Thus: $\log(OH^-)/(H^+) = pH - pOH = pH - pN + pN - pOH = 2(pH - pN)$.

Before the introduction of pH, two measures were used to indicate deviations from neutrality: the acidity, concerning the hydrogen ion concentration, and the alkalinity, concerning the hydroxyl ion concentration. The $(OH^-)/(H^+)$ ratio was therefore called the relative alkalinity by Rahn (31). Being equivalent to pH-pN, the $(OH^-)/(H^+)$ ratio is a superfluous quantity.

perature occurred. It is, nevertheless, possible that Austin and Cullen knew this paper.

Austin *et al.* (6) showed that in the alligator the $(OH^-)/(H^+)$ ratio was preserved after acclimatization to 9 °C and 35 °C. They found that the change in pH concomitant with changes in temperature was of such an extent that the net charge of plasma protein remained nearly constant. Later results obtained by Reeves and Wilson (34) indicated that the preservation of the protein net charge also applies to the intracellular protein of the frog skeletal muscle.

In addition to the results of Austin *et al.* (6), Robin (42), in 1962, found that in the blood of the turtle *in vitro*, of which the total CO_2 and O_2 contents were kept approximately constant, the changes in pH and pCO_2 with temperature were roughly the same as those *in vivo*. From this, Howell *et al.* (22) concluded that pH in ectotherms is regulated in such a way that the $(OH^-)/(H^+)$ ratio is preserved, *i.e.* that a constant difference between plasma pH and pN is maintained. Such a relationship between the pH of blood and pN could be caused by a buffer system of which the pK is about 7 and the $d(pK)/d(1/T)$ is about 1500, as was pointed out by Alberry and Lloyd (2). Reeves and Wilson (34), in their paper on the frog muscle, suggested that this buffer action would be exerted by the imidazole group of the histidine residues of proteins. In further work Reeves (35, 37) accumulated much material to strengthen the evidence that the imidazole group is indeed responsible for this property of proteins in blood and in cells. Reeves called this property the "imidazole alphastat" because of the preservation of the degree of ionization α of the imidazole group. In the ectotherms the regulation of arterial pH is executed by control of the ventilation leading to the proper pCO_2 (35).

The facts presented above apply to ectotherms in their adaptation to variations in the temperature of the environment. Yet another question is in how far the conclusions concerning normal pH and pCO_2 in ectotherms can be extrapolated to homeotherms under hypothermia. In addition to what is known about ectotherms, there is a rather convincing argument which is directly applicable to homeotherms. This argument is so straightforward that, in retrospect, one wonders why this argument was not used earlier. It short-circuits the whole detour over the ectotherms.

In the discussions about the influence of temperature on the blood gases and the acid-base status of the blood in homeotherms it was generally overlooked that homeotherms control their core temperature but that they are not isothermal, the extremities mostly being colder than the core of the body. It was only when it became clear that neither pH nor pCO_2 should be kept constant but rather pH-pN, and that this coincides with a constant composition of the blood on cooling, that a homeotherm's not being isothermal was recognized as being important for reaching conclusions about blood gases in hypothermia.

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In 1975 Rahn reviewed the results and insights gained by himself and his coworkers in his J. Burns Amberson Lecture (32). In that lecture he remarked that on its way to the periphery the blood coming from the core of the body may cool down by more than 20 °C. This cooling occurs while the blood is flowing through arteries in which virtually no exchange of substance takes place, so that the total O_2 and CO_2 contents remain the same. This cooling is, in fact, equivalent to the process Rosenthal (44) used when he determined the *in vitro* dependence of blood pH on temperature in a closed system. From this notion it follows that, at least for peripheral tissues, the composition of the arterial blood at temperatures other than 37 °C should be the same as that of normal blood arterialized at 37 °C, irrespective of the changes in temperature and the concomitant changes in pH, pCO_2, and pO_2. It is, of course, not certain whether a condition of the arterial blood well-suited for peripheral tissues is good for the internal organs as well. But with respect to this, the ectotherm argument is of just as little or even less value as the direct argument given above.

Contrary to the opinion expressed above about the probably optimal blood gas values for patients under hypothermia, other investigators argue that arterial pCO_2 should be kept constant when the temperature varies. This opinion is based on observations of blood gas values and acid-base control in hibernating animals during winter still active. In these animals pCO_2 can reach high levels and appears not to be very strictly controlled. A high pCO_2, however, leads to accumulation of CO_2 in the tissues, a situation not normally encountered in non-hibernating animals. At lower body temperatures even a constant arterial pCO_2 leads to an accumulation of CO_2.

In so far as the above questions concern the behaviour of blood under hypothermia, they are within the range of possibilities of the model developed earlier (52, 53). This model includes all the relevant temperature dependencies necessary to calculate blood gas and acid-base changes in human blood at temperatures other than 37 °C (310 K). The model was used to calculate the effects of two hypothetical policies of ventilation on blood gas and acid-base equilibria in arterial blood when its temperature is changed. One policy is that the total CO_2 content, tCO_2, and the total oxygen content, tO_2, are kept constant, the other that pCO_2 and tO_2 are kept constant. The quantities considered especially are plasma and red cell pH, pCO_2, hematocrit, donnan ratio, pO_2, and sO_2. The influence of hemoglobin content on the course of these quantities with temperature is also considered, as well as the influence of the initial tCO_2 and tO_2 on this course.

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THE MODEL

In the model the blood is assumed to consist of two compartments, each containing a homogeneous solution. The compartment representing plasma is assumed to contain a solution of NaCl, $NaHCO_3$, and protein in water. The other compartment, representing the red blood cells, is assumed to contain a solution of KCl, $KHCO_3$, ATP, 2,3-DPG, and hemoglobin in water. The two compartments are thought to be separated by a membrane, which is assumed to be impermeable to the kations, ATP, 2,3-DPG, hemoglobin, and plasma proteins. It is assumed to be permeable to O_2, CO_2, HCO_3^-, Cl^-, H^+, and water. The two compartments are in osmotic equilibrium. The model has been described in detail previously (52, 53).

Temperature influences practically all the equilibria in the blood. The influence on the solubility of CO_2 and on the various pK's have also been discussed in the earlier paper (52); that on the oxygen equilibrium curve of hemoglobin in a recent one (53). To calculate some of the results presented in this paper, the temperature dependences of two additional quantities were used: the solubility of oxygen in blood and the pH of pure water (pN).

To calculate changes in blood occurring when the oxygen content of the blood is constant and temperature is low, the amount of oxygen dissolved must be taken into account. The partitioning of oxygen between that combined with hemoglobin and that dissolved depends on the oxygen affinity of hemoglobin prevailing and on the solubility of oxygen in blood. The solubility of oxygen in blood is usually related to the solubility of oxygen in water of the same temperature. To calculate the latter a third degree polynomial was fitted through solubility values for water over the range 10-45 °C (283-318 K.) as given in (4). After transformation of these values to the quantities mmol, liter, and kPa the following expression results:

$$\alpha_{O2} = 10.75 \cdot 10^{-3} - 1.43 \cdot 10^{-4} \cdot (T - 308) + 3.30 \cdot 10^{-6} \cdot (T - 308)^2$$
$$- 20.2 \cdot 10^{-8} \cdot (T - 308)^3$$

Different findings have been published concerning the temperature dependence of the ratio between the solubilities of oxygen in water and in blood. The Biological Handbook (4) gives a relative increase in oxygen solubility in blood in proportion to that in water with decreasing temperature. Roughton and Severinghaus (43), instead, found a decrease in this ratio. Christoforides and Hedley-Whyte (17) and Hedley-Whyte and Laver (20) found a constant ratio between the two solubilities. They found that the solubility of oxygen in blood slightly depends on hemoglobin concentration. In this paper a constant

ratio of 0.935 between the solubility of oxygen in blood and that in water is used. This ratio is slightly higher than that given for plasma (16).

To calculate the values of pN at various temperatures, a second degree polynomial was fitted over the values of pN between 10 °C and 40 °C:

$$pN = 6.998 - 0.0167 \cdot (T - 298) + 8.5 \cdot 10^{-5} \cdot (T - 298)^2$$

CALCULATIONS

The temperature dependences of the relevant quantities were studied by calculating their equilibrium values with the model at five temperatures besides 37 °C: 42, 32, 27, 22, and 17 °C. Each calculation started with an initial condition defined at 37 °C. The initial conditions differed with respect to hemoglobin content, CO_2 content, and O_2 content. Calculations were done for blood with a hemoglobin content of 150, 50, 10, 0.5, and 0 g/L. The CO_2 content was varied by changing the pCO_2 at 37 °C. Three values were considered: 2.66, 5.33, and 10.66 kPa. To vary the intitial O_2 content four initial values of sO_2 were used: 0.98, 0.74, 0.50, and 0.26. In all, sixty different initial conditions were considered. For each case the course of the relevant quantities with temperature was calculated twice: once with pCO_2 and tO_2 constant and once with tCO_2 and tO_2 constant. In the latter series the equilibrium values of pO_2, sO_2 and pCO_2 at each temperature were calculated too.

The calculations were carried out on an HP 9845 A computer. The program was written in the extended version of BASIC implemented in this machine. It contained a number of iterative procedures. By controlled variation of some variables, the values were made to converge to an end state in which all the conditions set by the equations were satisfied to within 0.001 mmol/L.

RESULTS AND DISCUSSION

The results of the calculations are to a great extent given in the figures. Figure 1 shows the course of plasma pH with temperature when the total CO_2 content of blood with a hemoglobin concentration of 150 g/L and $pCO_2 = 5.33$ kPa and $sO_2 = 0.98$ at 37 °C is kept constant. The slope of the plasma pH curve is -0.0169 /K, that of the pN curve at 32°C -0.0156 /K, which shows that they are almost parallel to each other. In the literature several experimentally determined values of $\Delta pH/\Delta T$ have been reported: -0.017 (47), -0.0147 (44), -0.0140 (14), and ranging from -0.0137 to -0.0167 (7). The
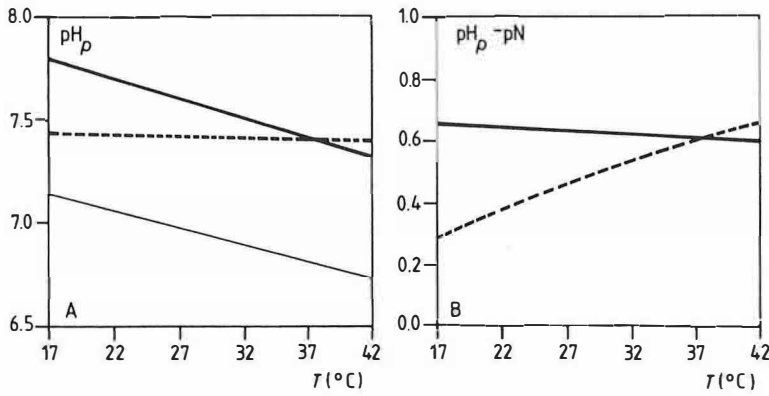
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Fig. 1. A. Influence of temperature on plasma pH in blood with 150 g/L hemoglobin in two conditions: tCO_2 constant at its value at 37 °C (thick line), and pCO_2 constant 5.33 kPa (broken line). The course of pN with temperature is given in addition (thin line). B. Plasma pH minus pN *versus* temperature. Same conditions as in A.

result that, when pCO_2 is kept constant, plasma pH hardly changes with temperature is also in agreement with experimental findings (13, 41).

In the case of constant tCO_2 the course of intra-erythrocytic pH is also almost parallel to both plasma pH and pN (figure 2). This agrees with the fact that at constant tCO_2 the donnan ratio hardly changes with temperature (figure 3A). At constant pCO_2 plasma pH remains nearly constant, but red cell pH slightly rises with decreasing temperature. This is caused by the rise in the donnan ratio. These results agree with those found by Reeves (36). The changes in the donnan ratio indicate the changes in the distribution of Cl^- and HCO_3^- between plasma and red cells. The exchanges of Cl^- and HCO_3^- lead to a new equilibrium in the distribution of water between plasma and red cells. This results in a change in the hematocrit with a change in temperature (figure 3B). This change does not occur when tCO_2 is constant.

The slopes of the curves calculated for plasma pH and red cell pH depend on the assumed initial conditions of the blood. They are affected by the hemoglobin concentration, the initial oxygen saturation and the initial pCO_2. The hemoglobin concentration has the greatest influence on the slope (figures 4 and 5). This is caused by the buffer capacity of hemoglobin, which is larger and more temperature dependent than that of plasma protein. This effect was experimentally studied by Rispens (39). His data points are widely scattered, possibly indicating that quantities other than those considered contribute to the temperature dependence of pH. The line fitted through his data and pivoting around the value of -0.0118 on the ordinate at zero hemoglobin concentra-
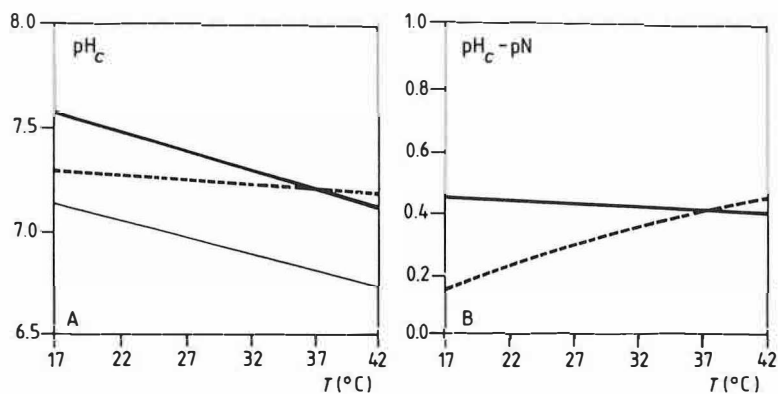
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Fig. 2. A. Influence of temperature on red cell pH in blood with 150 g/L hemoglobin in two conditions: tCO_2 is constant at its value at 37 °C (thick line), and pCO_2 is constant 5.33 kPa (broken line). Thin line: pN. B. Red cell pH minus pN *versus* temperature. Same conditions as in A.
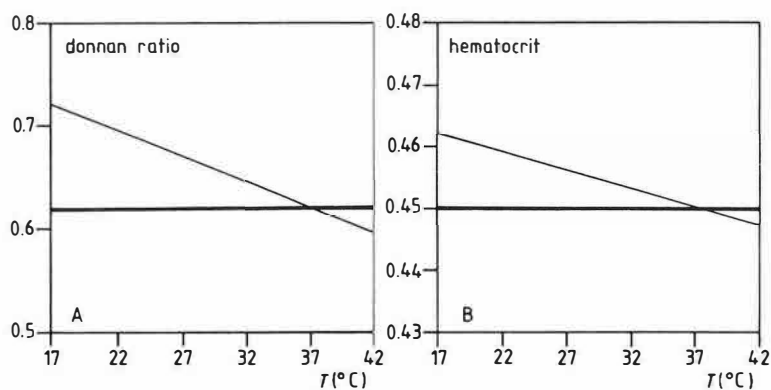

Fig. 3. Influence of temperature on the donnan ratio between red cells and plasma (panel A) and on the hematocrit (panel B) of blood with 150 g/L hemoglobin in two conditions: pCO_2 constant 5.33 kPa (thin lines) and tCO_2 constant at its value at 37 °C (thick lines). In both conditions tO_2 is kept constant at its initial value at 37 °C and $sO_2 = 0.98$.
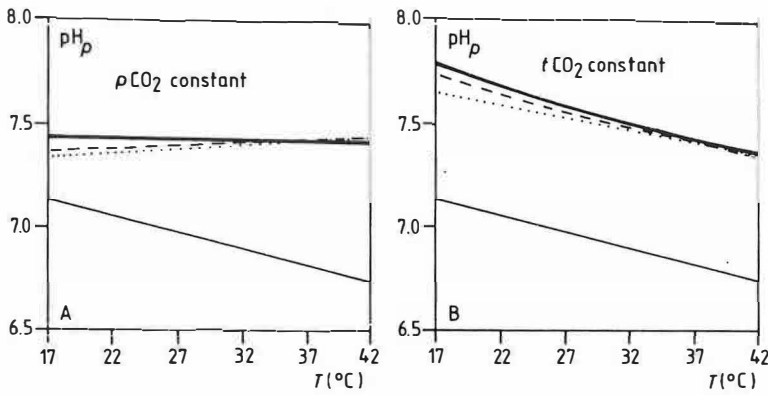
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Fig. 4. Influence of the hemoglobin content of blood on the temperature dependence of plasma pH in two conditions. Panel A: pCO_2 constant 5.33 kPa; panel B: tCO_2 constant at its value at 37 °C. Hemoglobin contents: 150 g/L (thick lines), 50 g/L (broken lines), 0.5 g/L (dotted lines). Thin lines: pN.

tion, *i.e.* plasma, gives the following relation between $\Delta pH/\Delta T$ and cHb:

$$\frac{\Delta pH}{\Delta T} = - (11.8 + 2.84 \cdot 10^{-2} \cdot cHb) \cdot 10^{-3}$$

Substitution of cHb = 150 g/L gives -0.0161 /K, while the slope of the cal-culated curve is -0.0169 /K. For cHb = 10 g/L the slope of the calculated curve is equal to that resulting from equation 3: -0.0121 /K. Adamson *et al.* (1) and Rispens (39) found that $\Delta pH/\Delta T$ is also dependent on the initial pH. Rispens found that it made no difference whether this pH was attained by titration with CO_2 or with non-volatile acid. Adamson *et al.* found that $\Delta pH/\Delta T$ increased by 0.005 per unit change in the initial pH; the results of Rispens lead to a value of 0.0058. This latter value was also found for the change in the slope of the calculated curves shown in figure 6B. In the calculations the change in the initial pH was attained by changing the intitial pCO_2 and oxygen saturation. Figures 6 and 7 give the curves for blood with a hemoglobin con-centration of 150 g/L, figures 8 and 9 those for "blood" with 0.5 g/L hemo-globin. The latter concentration is so small that the oxygen saturation no longer has a discernable effect on the plasma pH curves. Of course, there is an effect on the curves calculated for the red cell compartment (figure 9B).

The changes in pCO_2 and $\log(pCO_2)$ in blood with constant tCO_2 are shown in figure 10. For blood with 150 g/L hemoglobin, the slope of the curves of $\log(pCO_2)$ *versus* temperature between 27 and 37 °C is 0.023 /K. This value is in fair agreement with the experimental findings: 0.019 /K by Nunn *et al.*
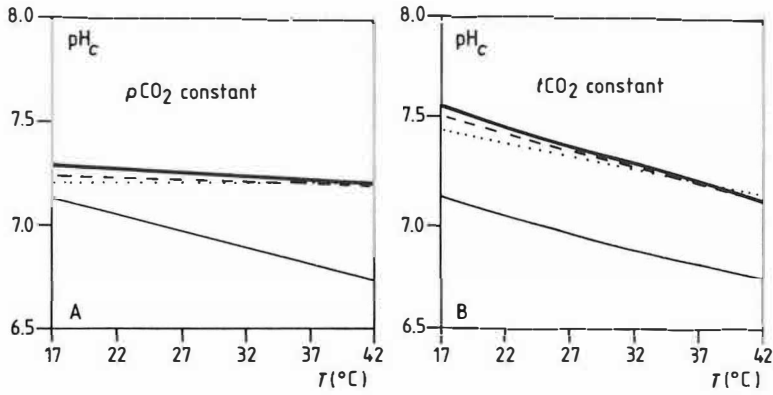
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Fig. 5. Influence of the hemoglobin content of blood on the temperature dependence of red cell pH in two conditions. Panel A: pCO_2 constant 5.33 kPa; panel B: tCO_2 constant at its value at 37 °C. Hemoglobin contents: 150 g/L (thick lines), 50 g/L (broken lines), 0.5 g/L (dotted lines). Thin lines: pN.

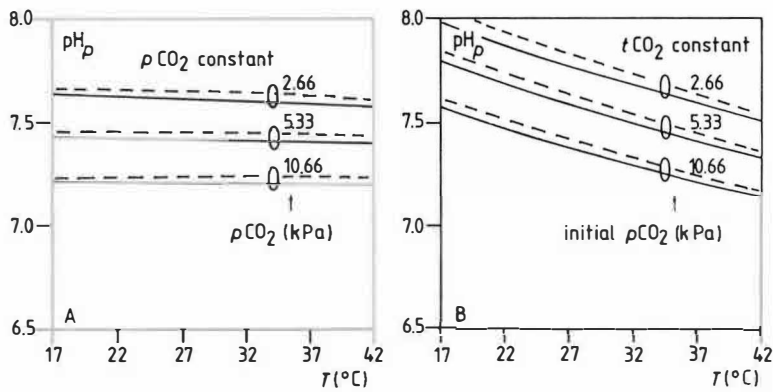Fig. 6. Influence of the oxygen saturation and the total carbon dioxide content at 37 °C on the temperature dependence of plasma pH in blood with 150 g/L hemoglobin in two conditions. Panel A: pCO_2 constant; panel B: tCO_2 constant at its value at 37 °C. Continuous lines: initial oxygen saturation 0.98; broken lines: initial oxygen saturation 0.26. The values of pCO_2 at 37 °C are given in the figure. In both conditions tO_2 is kept constant at its initial value at 37 °C.
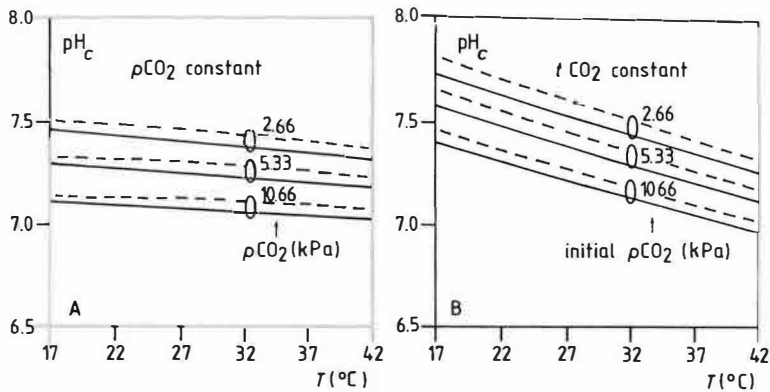
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Fig. 7. Influence of the oxygen saturation and the total carbon dioxide content at 37 °C on the temperature dependence of red cell pH in blood with 150 g/L hemoglobin in two conditions. Panel A: pCO_2 constant; panel B: tCO_2 constant at its value at 37 °C. Continuous lines: initial sO_2 0.98; broken lines: initial sO_2 0.26. The values of pCO_2 at 37 °C are given in the figure. In both conditions tO_2 is kept constant at its initial value at 37 °C.

(29), 0.021 by Castaing and Pocidalo (14), and 0.024 as derived from the nomogram by Rispens et al. (40). Bradley et al. (12) calculated 0.0185 /K for $\Delta \log(pCO_2)/\Delta T$. The pCO_2 calculated for blood at 20 °C is about 2 kPa. It follows from the course of pCO_2 with temperature (figure 10A) at several values of the hemoglobin concentration that in peripheral arterial blood the pCO_2 resulting after cooling only slightly depends on the hemoglobin concentration of the blood. Consequently, as long as the central pCO_2 is normal, it is the temperature in the peripheral tissues which determines the arterial pCO_2 in the peripheral tissues.

Figure 11 shows the effects of keeping either pCO_2 or tCO_2 constant on the amounts of the several forms in which CO_2 is present in the blood when the temperature varies. When pCO_2 is kept constant, the total amount of CO_2 increases to a considerable extent. The greater part of the increase is in the bicarbonate fraction, both in plasma and in the erythrocytes. When tCO_2 is kept constant (figure 11B), only slight changes in the partitioning of CO_2 appear. This is coherent with the constancy of the donnan ratio.

Figures 12 and 14 show the changes in the oxygen equilibrium with temperature when either pCO_2 or tCO_2 is kept constant. Figure 12 shows that except for very small hemoglobin concentrations, the changes in oxygen saturation do not differ much between the two policies. The changes in pO_2, however, are different in the two cases (figure 13). When pCO_2 is kept constant, the decrease in pO_2 is almost independent of the hemoglobin concentration (panel A), but with tCO_2 constant the decrease is clearly smaller at
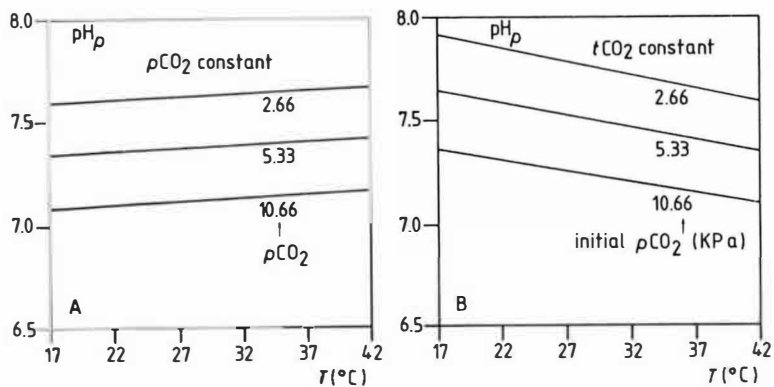
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Fig. 8. Influence of the oxygen saturation and the total carbon dioxide content at 37 °C on the temperature dependence of plasma pH in blood with 0.5 g/L hemoglobin in two conditions. Panel A: pCO_2 constant; panel B: tCO_2 constant at its value at 37 °C. The values of pCO_2 at 37 °C are given in the figure. In both conditions tO_2 is kept constant at its initial value at 37 °C. The curves with the initial $sO_2 = 0.98$ coincide with those of $sO_2 = 0.26$ because of the excess of plasma.
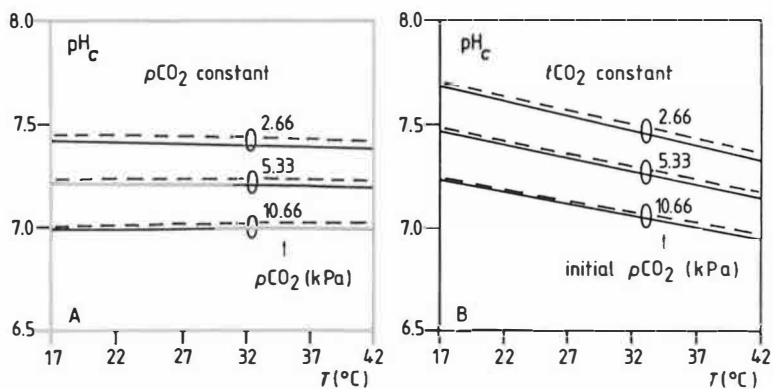
Fig. 9. Influence of the oxygen saturation and the total carbon dioxide content at 37 °C on the temperature dependence of red cell pH in blood with 0.5 g/L hemoglobin in two conditions. Panel A: pCO_2 constant; panel B: tCO_2 constant at its value at 37 °C. Continuous lines: initial sO_2 0.98; broken lines: initial sO_2 0.26. The values of pCO_2 at 37 °C are given in the figure. In both conditions tO_2 is kept constant at its initial value at 37 °C.

Fig. 10. Influence of hemoglobin content on the temperature dependence of pCO_2 (panel A) and $\log(pCO_2)$ (panel B) in blood of which tCO_2 and tO_2 are kept constant at their values at 37 °C with pCO_2 = 5.33 kPa and sO_2 = 0.98. Hemoglobin content 150 g/L (thick lines), 50 g/L (broken lines), 10 g/L (dotted lines), and 0 g/L (thin line).
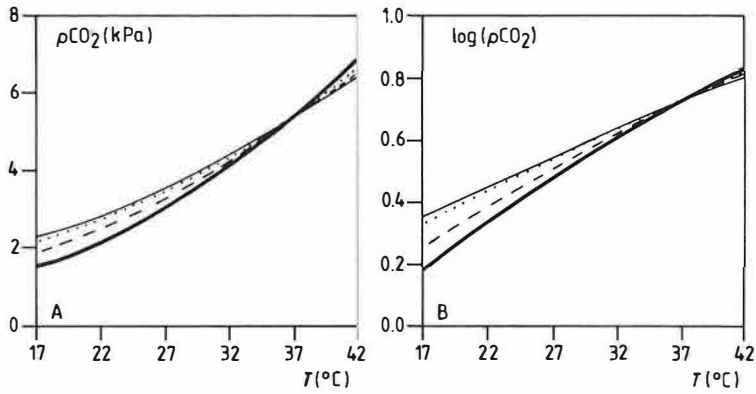
smaller hemoglobin concentrations. For the case with tCO_2 constant, Nunn *et al.* (29) found that the initial oxygen saturation had a marked influence. The slope of the $\log(pO_2)$ *versus* temperature curve was 0.0187 /K at sO_2 = 0.98, 0.0293 /K at sO_2 = 0.91, and 0.032 /K at sO_2 = 0.83. The calculated curves were also clearly influenced by the initial sO_2, but this influence was greatly dependent on the hemoglobin concentration. In blood with cHb = 150 g/L the calculated slope is 0.033 /K at sO_2 = 0.98, and 0.034 /K at sO_2 = 0.74 and 0.50. In blood with cHb = 50 g/L this slope is 0.020 /K at sO_2 = 0.98, 0.031 /K at sO_2 = 0.74, and 0.032 /K at sO_2 = 0.50. In blood with cHb = 10 g/L the slope is 0.0096 /K at sO_2 = 0.98, 0.028 at sO_2 = 0.74, and 0.029 at sO_2 = 0.50. At cHb = 50 g/L our calculated values agree reasonably well with those found by Nunn *et al.* (29); they, however, report a hemoglobin concentration of 150–180 g/L.

Figure 14 shows the changes in the logarithm of the median oxygen tension, $\log(p_m)$, (53), with temperature. As far as changes are concerned, p_m can be considered equivalent to p_{50}, the oxygen tension at sO_2 = 0.5. Panel B shows the changes when tCO_2 is constant. The value calculated from the curve with cHb = 150 g/L is 0.034 /K. From the experimental data of Reeves (38) follows that in blood with constant tCO_2 the slope of $\log(p_{50})$ *versus* temperature is 0.032 /K. The experiments reported in (54) and (55) yield a value of 0.033 /K. Castaing and Sinet (15) have measured this quantity in blood with 7.5 g/L hemoglobin. They found a value of 0.027 /K. The calculated curve of $\log(p_m)$ *versus* temperature for blood with 10 g/L hemoglobin has a slope of 0.030 /K. In both cases, the calculated values are slightly

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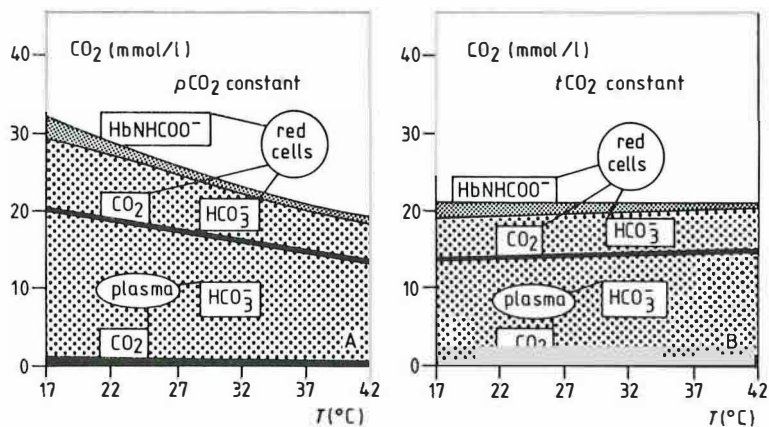
Fig. 11. Influence of temperature on the total carbon dioxide content and its partitioning in blood with 150 g/L hemoglobin in two conditions. Panel A: pCO_2 is constant at 5.33 kPa; panel B: tCO_2 constant its value at 37 °C with pCO_2 = 5.33 kPa. In both conditions tO_2 is kept constant at its value at 37 °C with sO_2 = 0.98.
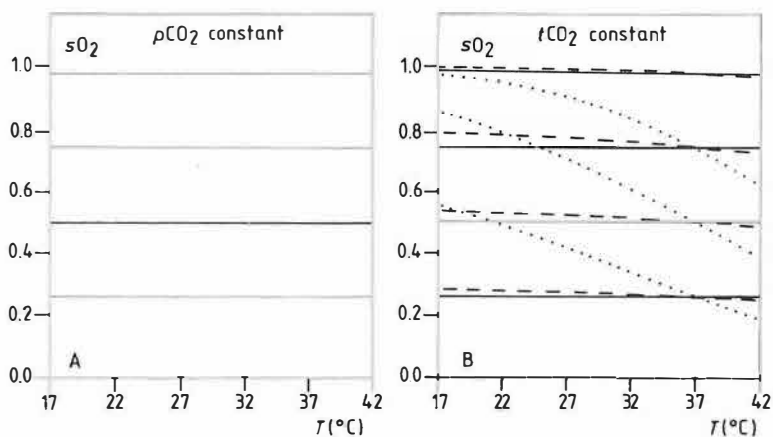
Fig. 12. Influence of hemoglobin content on the temperature dependence of the oxygen saturation in two conditions. In both conditions the total oxygen content tO_2 is kept at its value at 37 °C. Panel A: pCO_2 constant 5.33 kPa. Each curve represents three values of the hemoglobin content: the three lines coincide. Panel B: tCO_2 constant at its value at 37°C with pCO_2 5.33 kPa. Hemoglobin content: 150 g/L (continuous lines), 10 g/L (broken lines), and 0.5 g/L (dotted lines).

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Fig. 13. Influence of hemoglobin content on the temperature dependence of the oxygen tension pO_2 in blood in two conditions. In both conditions the total oxygen content tO_2 is kept at its value at 37 °C. Panel A: pCO_2 constant 5.33 kPa; panel B: tCO_2 constant at its value at 37 °C with pCO_2 5.33 kPa. Hemoglobin content: 150 g/L (thick lines), 50 g/L (broken lines)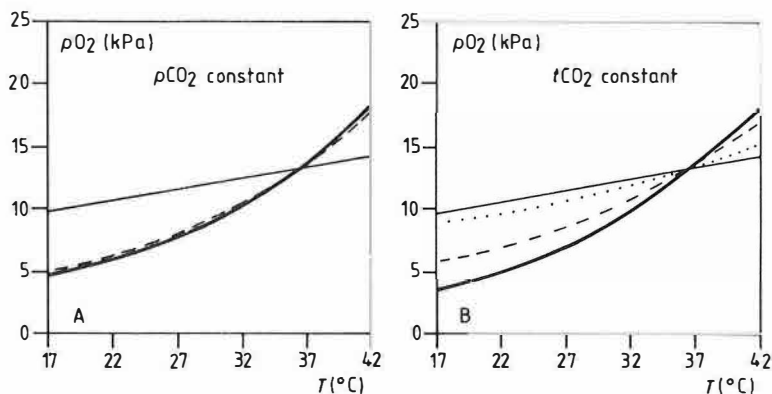, 10 g/L (dotted lines), and 0 g/L (thin lines). In panel A the dotted line cannot be distinguished from the broken line.

higher than the experimental ones.

Overall, the values calculated for blood gas and acid-base quantities agree fairly well with values found experimentally. This indicates that the model used offers a good representation of the equilibria that determine blood gas and acid-base parameters of blood. This also includes the oxygen equilibria, the calculations of which are based on the relations between the H^+ and CO_2 binding properties of hemoglobin and the concomitant changes in $\log(p_m)$ (53). In the model the carbon dioxide and oxygen equilibria are treated in a coherent and consistent way. As far as the temperature dependence of oxygen equilibria other than those pertaining to the OEC is concerned, experimental data are scarce and this subject deserves further attention.

The question of how to interpret blood gas and acid-base values at other temperatures has not been settled yet (19, 33). This paper cannot provide conclusive arguments for the use of a specific procedure, but it shows that complex interactions should be taken into account when calculating from 37 °C to patient temperature. This complexity is not duly considered in most cases and rules of thumb are used. But even conversion factors (7), nomograms (24, 27, 40) or algorithms (49) are not reliable as long as they do not consider all possible relations in blood in combination with all the relevant concentrations, which, however, is virtually impossible. Moreover, the fact that every acid-base status of the blood at any temperature in the physiological range has its unique representation at 37 °C allows the assessment of the acid-base status of
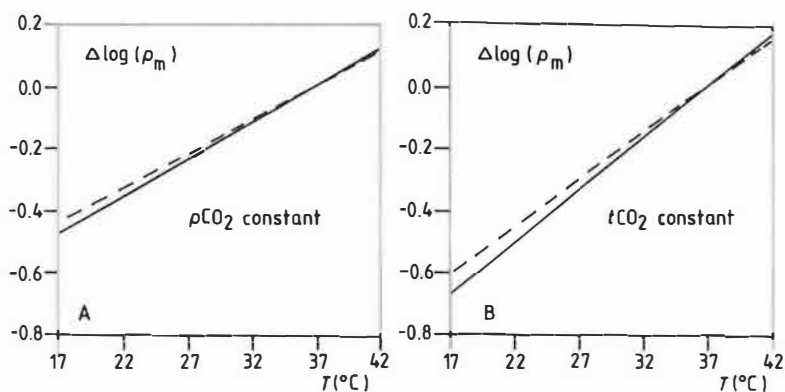
Fig. 14. Influence of hemoglobin content on the temperature dependence of the change in $\log(\rho_m)$ in blood in two conditions. In both conditions the total oxygen content is kept at its value at 37 °C. Panel A: pCO_2 constant 5.33 kPa; panel B: tCO_2 constant at its value at 37 °C with pCO_2 5.33 kPa. Hemoglobin content 150 g/L (continuous lines) and 0.5 g/L (broken lines).

blood from measurements at 37 °C unambiguously and makes conversion to bôdy temperature superfluous. To prevent confusion, blood gas and acid-base values should be measured at and reported for 37 °C.

The policy to be adopted concerning the arterial pCO_2 in patients under hypothermia cannot be determined on the basis of the study of the blood gases and acid-base equilibria in blood *in vitro*. This study shows the differences in blood gas values and acid-base equilibria resulting from the two policies of ventilation used. The fact that no major changes occur when tCO_2 is kept constant is a plea in favour of this policy if this would turn out to be the case not only in blood but in other tissues as well. The body of evidence showing that this is in fact the case appears to be growing. Confirmation is found in experiments on animals (8, 26, 30) as well as in clinical situations where surgical intervention takes place in patients under hypothermia (10).

ACKNOWLEDGEMENT

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CHAPTER V

CARBON DIOXIDE LOADING AND THE ACID-BASE
EQUILIBRIUM STATES OF THE HUMAN ISOLATED
INTERNAL ENVIRONMENT

Carbon dioxide loading and the acid-base equilibrium states of the human isolated internal environment*

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ABSTRACT

The changes in plasma pH and plasma bicarbonate concentration accompanying changes in pCO_2 are different for blood *in vivo* and blood *in vitro*. This difference is caused by buffering and dilution in the interstitial fluid, by the effects exerted by the tissue cells and the gut, and by the regulatory actions of the kidneys. Because of the intricate intermingling of the interstitial fluid with the tissue cells it is not possible to separate experimentally the contribution of the interstitial fluid to and the effects of the tissue cells on the CO_2 bufferline *in vivo*. In order to get an impression of the influence of the interstitial fluid on the *in vivo* buffering, a model was developed with which equilibrium states of the blood of the body in venous composition with all the interstitial fluid can be calculated. The model consists of a number of equations describing, first, the relevant physico-chemical properties of the homogeneous solutions contained in the three supposed compartments (red cells, plasma and interstitial fluid) and, second, the equilibrium conditions on the two boundaries between the compartments (red cell-plasma and plasma-interstitial fluid). The plasma compartment is the central one, in equilibrium on one side with the erythrocyte content, and on the other side with the interstitial fluid. The equilibrium conditions and properties of the blood have been described previously (28). The interstitial fluid was assumed to reach equilibrium with plasma through exchange of all its supposed constituents except protein. This condition, together with the need to assign an arbitrary value to the volumes of the compartments, necessitated the introduction of a hydrostatic pressure difference between the blood and the interstitial fluid compartment. The model was implemented as a computer program. The results as calculated with this model can, however, only indirectly be compared with measured values since the interstitial fluid is not accessible to an experimental approach.

Cells in the body are surrounded by the interstitial fluid. This fluid forms the environment from which the cells are supplied with nutrients and into which they dispose of the end products of their metabolism. Both the uptake of nutrients and the disposal of end products tend to change the composition of the interstitial fluid. For optimal functioning, cells require a well adjusted composition of the surrounding interstitial fluid. This composition is maintained by supply from and disposal into the blood. Constancy of the composition of the blood is attained by regulatory actions of the body. Interstitial fluid is a filtrate of blood plasma and by diffusion most of its constituents exchange very rapidly with plasma (19). In this way the composition of the interstitial fluid is related to the composition of blood plasma and, as far as oxygen and carbon dioxide are concerned, also to the composition of whole blood. The extracellular fluid (*i.e.* interstitial fluid and blood plasma) and the erythrocytes together constitute the internal environment supplying the tissue cells. Although erythrocytes are living cells too, they are considered as being part of the internal environment because their functions in the maintenance of the constancy of the internal environment are operative throughout the body in close interaction with plasma, at a very short distance from the interstitial fluid.

One of the important variables representing the state of the interstitial fluid is pH. This is partly determined by the balance between the production and the removal of CO_2, which, besides water, is the main end product of oxidative cell metabolism. In the body CO_2 is present as dissolved CO_2, as HCO_3^- ions, and, bound to proteins − mainly hemoglobin − , as carbamate. It is, furthermore, a constituent of bone in which it is complexly bound. As such it only participates in very slow exchanges of CO_2 and is usually not considered in this context (11). The amount of dissolved CO_2 depends on the solubility and the partial pressure of CO_2. In the tissues the latter is the result of the partial pressure of CO_2 in arterial blood, the perfusion and the rate of production of CO_2. With the formation of HCO_3^- ions and also with carbamate formation, protons are released which are buffered both inside and outside the tissue cells. Inside the tissue cells the main buffers are phosphates and proteins, while outside the tissue cells these buffers are hemoglobin in the red cells and the proteins of plasma and interstitial fluid. Plasma is in very close contact with interstitial fluid and the acid-base status of venous plasma shortly after the exchange in the tissue reflects the acid-base status of the perfused tissue.

Besides the influence of CO_2 on the acid-base status of the interstitial fluid, that of non-volatile ionic substances is also important. Abnormal concentrations of these can result from metabolism, as *e.g.* lactic acid in hypoxia, as well as from disturbances in the balance between uptake and excretion of electrolytes, as *e.g.* in kidney failure or after profuse vomiting or diarrhoea.

Acid-base disturbances which follow a period of imbalance between production and removal of CO_2 are caused by inadequate respiration and are thus called *respiratory* disturbances, while all others are called *non-respiratory*

disturbances. Disturbances of overall acid-base status can be best detected in the plasma of arterial blood, because the composition of this blood is subjected to the regulatory action of the body as effected in the lungs. Any deviation in the acid-base status remaining after passage of the blood through the lungs indicates a disturbance of homeostasis.

In isolated plasma of constant temperature which is equilibrated with a gas mixture containing CO_2, the acid-base status is characterized by two of the three variables: pCO_2, pH and plasma bicarbonate concentration (c_{p, HCO_3^-}). The third variable is related to the other two by the Henderson-Hasselbalch relation (Eq. 9). This plasma is called "separated plasma". Its acid-base status can be unambiguously represented as a point in a diagram with two of the variables plotted along the axes. Varying the pCO_2 is equivalent to titration of the plasma with carbonic acid. The line in the $c_{p, HCO_3^-}/pH_p$ diagram which is found when varying the pCO_2, is called the bufferline of separated plasma. The buffer value is the slope of this line and depends upon the buffering by non-bicarbonate buffers.

When whole blood of constant temperature is equilibrated with a gas mixture containing O_2 and CO_2, the buffering by the hemoglobin inside the erythrocytes and the redistribution of HCO_3^- and Cl^- across the red cell membrane influences the acid-base status of the plasma. This results in a steeper bufferline than that of separated plasma. Such plasma is called "true plasma *in vitro*". The oxygen saturation of hemoglobin has some influence on the position of the bufferline, that of deoxygenated normal blood lying about 3 mmol·l^{-1} above that of fully oxygenated blood.

Plasma circulating in the body as constituent of the blood is called "true plasma *in vivo*". This plasma is in direct contact with erythrocytes as well as with the tissues. Tissue cells can readily exchange substances with plasma by way of the interstitial fluid. Because of this, the acid-base status of mixed venous plasma reflects the average acid-base status of the various tissues of the body. By changing the balance between removed and produced CO_2, the whole body can be titrated with carbonic acid. The result of this titration is represented in the bufferline as found for mixed venous plasma. Apart from the buffering by the internal environment, the slope of this bufferline depends on shifts of electrolytes between the internal environment and the tissue cells, on the buffering which takes place inside the tissue cells, on the production and utilization of organic ions and on renal regulations. The quantitative assessment of these contributions to the buffering in the body requires that they be distinguished from the buffering by the internal environment alone. Experimentally, this separation cannot be realized. To cope with this difficulty the existing model of blood *in vitro* (28) was extended by the addition of the interstitial fluid, thus forming a model of the "isolated internal environment" *i.e.* the blood in exchange with the interstitial fluid.

The model consists of a set of equations describing the behaviour of the isolated internal environment under the assumption of a number of simplifying idealizations. It opens up the possibility of calculating to what extent buffering

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takes place by the internal environment and thus to estimate the contributions of other influences on the bufferline of true plasma *in vivo*.

MODEL

If the body could be in a completely steady state there would be a balance between production and removal of CO_2, local tissue concentrations would not vary with time and the composition of arterial and venous blood would be constant. In the model a completely steady state was assumed. It was further-more assumed that diffusion and the reactions and processes related to CO_2 transport are so fast that in this steady state the deviations from equilibrium could be neglected. This steady state of an open system is then identical with an equilibrium state of a closed system.

In the model the internal environment consists of all the blood of the body as modelled by Zock *et al.* (28) in a venous state in equilibrium with all the inter-stitial fluid of the body across a selectively permeable capillary wall (fig. 1). Blood is a suspension of erythrocytes in plasma. Plasma is assumed to be a homogeneous solution of NaCl, $NaHCO_3$ and proteins in water. The red cells consist of a selectively permeable membrane containing the red cell content and separating it from the plasma. Red cell content is assumed to consist of a homogeneous solution of KCl, $KHCO_3$, ATP, 2,3-DPG and hemoglobin in water. Interstitial fluid is assumed to be a homogeneous solution of NaCl, $NaHCO_3$ and plasma protein. Na^+ represents all kations of plasma and inter-
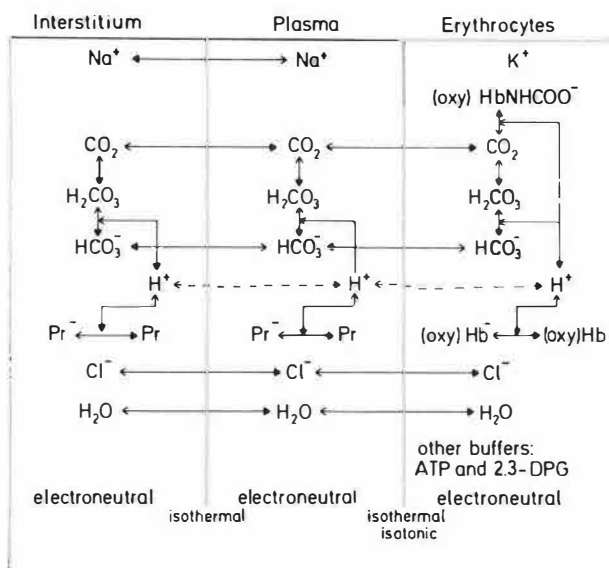

Fig. 1. Schematic representation of the equilibria considered in the model of the isolated internal environment. Closed arrows indicate the direction in which the equilibria shift when CO_2 is added; open arrows indicate the shift when CO_2 is withdrawn.

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stitial fluid, K^+ stands for all kations present in the erythrocytes. K^+ is assumed to be confined to the erythrocyte compartment, Na^+ to stay completely out of it.

The red cell membrane is taken to be impermeable to Na^+, K^+, plasma proteins, hemoglobin, ATP and 2,3-DPG, and freely permeable to CO_2, H_2O, Cl^- and HCO_3^-. This means that at equilibrium the erythrocytes are in osmotic equilibrium with plasma. In the model the red cell membrane has no other properties. The property ascribed to the capillary wall is that it is impermeable to proteins, letting all other substances exchange freely between plasma and interstitial fluid.

The oxygen saturation of hemoglobin is given a certain value. The amount of dissolved oxygen is neglected. In the model it is assumed that the temperature is the same throughout the isolated internal environment.

The electroneutrality of the three solutions — plasma, erythrocyte content and interstitial fluid — requires that

(1) $n_{p,\,Na^+} = n_{p,\,Cl^-} + n_{p,\,HCO_3^-} + z_{Pr} \cdot w_{p,\,Pr}$

(2) $n_{c,\,K^+} = n_{c,\,Cl^-} + n_{c,\,HCO_3^-} + z_{ATP} \cdot n_{c,\,ATP} + z_{DPG} \cdot n_{c,\,DPG} +$
       $+ z_{Hb} \cdot n_{c,\,Hb} + z_{HbO_2} \cdot n_{c,\,HbO_2}$

(3) $n_{i,\,Na^+} = n_{i,\,Cl^-} + n_{i,\,HCO_3^-} + z_{Pr} \cdot w_{i,\,Pr}$

where n denotes an amount of substance in mole, w a mass in gram. The index p means plasma, c is red cell, i stands for interstitial fluid; z is the amount of charge per mole, except for plasma and interstitial protein where it is the amount of negative charge per gram protein.

Cl^- and HCO_3^- are assumed to be distributed between plasma water and red cell water according to the Gibbs-Donnan equilibrium conditions:

(4) $r_{c,p} = \dfrac{c_{cw,\,Cl^-}}{c_{pw,\,Cl^-}} = \dfrac{c_{cw,\,HCO_3^-}}{c_{pw,\,HCO_3^-}}$

$r_{c,p}$ is the Donnan ratio and c_{cw} and c_{pw} denote the substance concentration in cell water and plasma water, respectively. The ratio of proton activity a_{H^+} in plasma water and in red cell water is also assumed to be according to the Gibbs-Donnan condition, so that

(5) $\log(r_{c,p}) = pH_c - pH_p$.

The supposed distribution of Na^+, Cl^- and HCO_3^- between plasma water and the water of the interstitial fluid according to the Gibbs-Donnan condition gives:

(6) $r_{i,p} = \dfrac{c_{iw,\,Cl^-}}{c_{pw,\,Cl^-}} = \dfrac{c_{iw,\,HCO_3^-}}{c_{pw,\,HCO_3^-}} = \dfrac{c_{pw,\,Na^+}}{c_{iw,\,Na^+}}$

where c_{iw} is the substance concentration in interstitial water. Applied to a_{H^+} this gives

(7) $\log(r_{i,p}) = pH_i - pH_p$.

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The conditions of osmotic equilibrium between erythrocytes and plasma and the balance between osmotic and hydrostatic pressures between blood and interstitium leads to

$$
(8) \quad
\begin{cases}
\phi_e \cdot RT \cdot \left(\dfrac{n_{i,\mathrm{Na}^+} + n_{i,\mathrm{Cl}^-} + n_{i,\mathrm{HCO_3}^-}}{w_{i,\mathrm{H_2O}}} \right) + \pi_{i,\mathrm{Pr}} + \Delta p_h = \\[2ex]
= \phi_e \cdot RT \cdot \left(\dfrac{n_{p,\mathrm{Na}^+} + n_{p,\mathrm{Cl}^-} + n_{p,\mathrm{HCO_3}^-}}{w_{p,\mathrm{H_2O}}} \right) + \pi_{p,\mathrm{Pr}} = \\[2ex]
= \phi_e \cdot RT \cdot \left(\dfrac{n_{c,\mathrm{K}^+} + n_{c,\mathrm{Cl}^-} + n_{c,\mathrm{HCO_3}^-} + n_{c,\mathrm{ATP}} + n_{c,\mathrm{DPG}}}{w_{c,\mathrm{H_2O}}} \right) + \pi_{c,\mathrm{Hb_4}}
\end{cases}
$$

where ϕ_e is the osmotic activity coefficient of the electrolytes, $\pi_{i,\mathrm{Pr}}$ the osmotic pressure of the protein in the interstitial fluid, $\pi_{p,\mathrm{Pr}}$ the osmotic pressure of plasma protein, $\pi_{c,\mathrm{Hb_4}}$ the osmotic pressure of hemoglobin, $w_{i,\mathrm{H_2O}}$, $w_{p,\mathrm{H_2O}}$, $w_{c,\mathrm{H_2O}}$ the masses of interstitial, plasma and cell water, and Δp_h the difference in hydrostatic pressure between capillary lumen and the interstitium necessary to get equilibrium under the conditions imposed by the other relations mentioned, while still allowing the freedom to choose the volumes of the three compartments arbitrarily. The relation between plasma pH, $p\mathrm{CO_2}$ and plasma $\mathrm{HCO_3}^-$ concentration is given by the Henderson-Hasselbalch equation:

$$
(9) \quad \mathrm{pH}_p = \mathrm{p}K_1' + \log \frac{c_{p,\mathrm{HCO_3}^-}}{S' \cdot p\mathrm{CO_2}}
$$

where $c_{p,\mathrm{HCO_3}^-}$ is the plasma $\mathrm{HCO_3}^-$ concentration in $\mathrm{mmol \cdot l^{-1}}$ and S' the solubility of $\mathrm{CO_2}$ in plasma in $\mathrm{mmol \cdot l^{-1} \cdot kPa^{-1}}$.

The temperature dependences of $\mathrm{p}K_1'$ and S' are according to Rispens (22):

$$
(10) \quad \mathrm{p}K_1' = -4.7416 + 1840.11/T + 0.015906T - \log \left(1 + \frac{0.020682}{10^{(7 - \mathrm{pH}_p(T))}} \right)
$$

and

$$
(11) \quad S' = 7.50(-3.246 + 536.73/T + 0.004985T)
$$

where T is temperature in K. Provided that suitable expressions are available for the titration curves of the proteins, hemoglobin, ATP and 2,3-DPG and that the osmotic activity coefficients and the relation between protein and water content of a solution are given, the above equations with proper starting values are sufficient for calculating the interdependence of the many variables such as oxygen saturation ($S_{\mathrm{O_2}}$), carbon dioxide tension ($p\mathrm{CO_2}$), plasma pH (pH_p), $\mathrm{CO_2}$ content of the internal environment, the slope of the bufferline of true plasma *in vivo* and temperature (T_b).

The titration curve of plasma protein at 37 °C is given by

$$
(12) \quad z_{p,\mathrm{Pr}} = 0.107 \, (\mathrm{pH}_p - 5.10).
$$

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The slope of this curve is commonly assumed to be independent of temperature. We used a change in iso-electric pH of -0.014 pHu$\cdot$K^{-1}, according to Rispens (22).

The titration curve of hemoglobin depends on oxygen saturation, carbon dioxide tension, 2,3-DPG concentration and temperature (18). The influences of oxygenation and carbon dioxide binding on the titration curve of hemoglobin are treated as independent effects, superimposed upon the titration curve of deoxygenated hemoglobin in the absence of CO_2. At 37 °C the amount of charge on one mole of Hb monomer is fairly approximated by

$$(13) \qquad z_{Hb} = 2.7\,(pH_c - 6.81).$$

The temperature dependence of the slope is -0.009 mmol$\cdot$pHu$^{-1}\cdot$K^{-1} and the iso-electric point changes by -0.0122 pHu$\cdot$K^{-1} (1).

The titration curve of oxygenated hemoglobin differs slightly from that of deoxygenated hemoglobin. This is caused by changes in equilibrium constants of alkaline and acidic Bohr groups of the hemoglobin molecule which occur with oxygenation (1, 18, 25), each group thus being characterized by two constants. The difference in charge between one mole deoxygenated and one mole oxygenated hemoglobin is calculated with the use of these constants. They are for the acidic group $pK_{Hb} = 5.4$ and $pK_{HbO_2} = 5.9$, for the alkaline group $pK_{Hb} = 7.5$ and $pK_{HbO_2} = 6.45$. The temperature dependence of the alkaline group is -0.0265 K^{-1} and -0.0167 K^{-1}, respectively, and that of the acidic Bohr groups is 0.004 K^{-1} (1, 18).

The binding of CO_2 by hemoglobin influences the titration curve of hemoglobin, because an alkaline amino group is changed into an acid carbamino group. From the equations describing these reactions, it follows that (24)

$$(14) \qquad Z = \frac{K_c' \cdot K_z' \cdot c_{c,CO_2}}{K_c' \cdot K_z' \cdot c_{c,CO_2} + K_z' \cdot a_{H^+} + (a_{H^+})^2}$$

where Z is the CO_2 saturation of hemoglobin, c_{c,CO_2} the concentration of dissolved CO_2 (mol$\cdot$l^{-1}) and a_{H^+} the proton activity (mol$\cdot$l^{-1}). The equilibrium constants K_c' and K_z' are different for oxygenated and deoxygenated hemoglobin. Partially oxygenated hemoglobin is considered to be a mixture of fully saturated and completely deoxygenated hemoglobin, mutually independent. The values of the constants used are $pK_{c,Hb} = 4.6$, $pK_{c,HbO_2} = 5.0$ with temperature coefficient 0.019 K^{-1} and $pK_{z,Hb} = 7.0$, $pK_{z,HbO_2} = 6.5$ with temperature coefficient -0.022 K^{-1}.

The concentration of dissolved CO_2 in the erythrocytes was calculated using a solubility of 0.195 mmol$\cdot$l$^{-1}\cdot$kPa^{-1} at 37 °C (28). The temperature variation of this value was assumed to be in proportion with the temperature variation of the CO_2 solubility in plasma.

The charges on ATP and 2,3-DPG as represented by z_{ATP} and z_{DPG} were calculated with $pK' = 7.04$ and $dpK'/dT = -0.0124$ for ATP and with $pK' = 6.73$ and $dpK'/dT = 0.0026$ for 2,3-DPG (21).

The osmotic coefficient for the ions was supposed to be 0.92. The colloid osmotic pressure of plasma proteins was taken as

(15) $\quad \pi_{plasma} = 0.028 \, c_{p, Pr} + 2.133 \; 10^{-4} \, c^2_{p, Pr} + 1.200 \; 10^{-6} \, c^3_{p, Pr}$

where π is the pressure in kPa and $c_{p, Pr}$ is the plasma protein concentration in $g \cdot l^{-1}$. The equivalent expression used for the colloid osmotic pressure of hemoglobin was

(16) $\quad \pi_{Hb_4} = 0.040 \, c_{Hb_4} + 2.57 \; 10^{-4} \, c^2_{Hb_4} + 1.71 \; 10^{-6} \, c^3_{Hb_4}.$

The volume occupied by 1 g of hemoglobin and that by 1 g of protein was assumed to be 0.85 ml. This volume was assumed to include the volumes occupied by non-hemoglobin proteins in the erythrocytes and by lipids in plasma and interstitial fluid (28).

ISOLATED INTERNAL ENVIRONMENT (IIE)

The equations given above interrelate the variables; because of this only a restricted number of variables can be chosen independently. The values which are chosen determine the composition and the state of the isolated internal environment. The interstitial fluid is considered to be in equilibrium with venous blood plasma, the composition of venous blood being calculated from arterial blood. Standard arterial blood is determined by assuming the following

Table I. Calculated composition of venous blood at RQ = 0.83, compared with arterial blood.

Quantity	Unit	Arterial blood			Venous blood		
		Plasma	Erythro-cytes	Blood	Plasma	Erythro-cytes	Blood
S_{O_2}			1 *			0.76 *	
pCO_2	kPa	5.33 *	5.33 *		6.13 *	6.13 *	
pH	pHu	7.410*	7.202*		7.378	7.184	
$r_{c,p}$				0.619			0.638
Total O_2	mmol		9.31	9.31		7.07	7.07
Total CO_2	mmol	14.36	6.89	21.25	15.31	7.81	23.11
Dissolved CO_2	mmol	0.67	0.47	1.14	0.77	0.54	1.31
Carbamate	mmol		1.13	1.13		1.42	1.42
HCO_3^-	mmol	13.69	5.29	18.98	14.53	5.85	20.38
Cl^-	mmol	60.90	23.58	84.48	60.20	24.28	84.48
Charge of protein	mmol	9.52			9.39		
Charge of hemoglobin	mmol		15.47			14.24	
Charge of DPG	mmol		7.93			7.90	
Charge of ATP	mmol		1.74			1.73	
Volume	l	0.550	0.450	1.000*	0.548	0.452	1.000 *
Water	l	0.517	0.323	0.840	0.515	0.325	0.840
Osmolality	mosm/kg	286.1			287.5		
Colloid osmotic pressure	kPa	3.44	106.9		3.46	105.6	
Total osmotic pressure	kPa	736.8	736.8		740.6	740.6	

* Assumed values.

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values: $T = 310$ K, $S_{O_2} = 1$, $pCO_2 = 5.33$ kPa (40 mmHg), $pH_p = 7.410$, $pH_c = 7.202$, $c_{p, Na^+} = 153$ mmol$\cdot$l^{-1}, $c_{p, Pr} = 70$ g$\cdot$l^{-1}, $c_{b, Hb} = 150$ g$\cdot$l^{-1}, $c_{c, Hb} = 333$ g$\cdot$l^{-1} and $c_{cw, ATP} = 1.5$ mmol$\cdot$l^{-1}. To calculate the composition of venous plasma it was assumed that in the venous blood $S_{O_2} = 0.76$ and the respiratory quotient is 0.83 with respect to standard arterial blood, which corresponds in the model with a $pCO_2 = 6.13$ kPa. The calculated amounts of the substances in arterial and venous blood are given in table I. Additional values needed to fix the remaining degrees of freedom are the concentration of protein in the interstitial fluid and the volumes of blood and interstitial fluid. The concentration of protein in the interstitial fluid was assumed to be 35 g$\cdot$l^{-1} (4). The volumes were based on the assumptions that the volume of the extracellular water is 200 ml per kg of body mass and that blood volume is 76 ml per kg of body mass. These generally accepted values applying to young men were used to calculate the volumes of blood and interstitial fluid for the "physiological standard man", which means a young man of 70 kg body mass.

CALCULATIONS

The above-mentioned equations were implemented in a computer program. Values for the quantities which can be chosen independently were provided in order to calculate the composition of the isolated internal environment. When the above-mentioned values were put into the program the standard composition followed. The insertion of various values of the independent quantities makes it possible to study their effects. The variation of one or more of the variables defining the state of the system, *e.g.* like pCO_2, S_{O_2} and temperature, results in relations between the variables of the system. One of our main interests was in the effect of the titration of the isolated internal environment with CO_2. This titration results in a change in plasma pH and plasma bicarbonate concentration. A plot of these two quantities is the CO_2 bufferline. Other relations can be calculated in a similar way. The results of these calculations are presented in the next section.

The equations were solved by consecutive approximations. The maximum permitted error in the solutions was 0.0001 pHu. The approximation was stopped when the conditions set by the equations were satisfied to the chosen precision.

RESULTS

The values of a number of quantities as calculated with the IIE model for standard conditions and standard composition are given in table II. The Donnan distribution of Na$^+$, HCO$_3^-$, Cl$^-$ and H$^+$ between plasma and interstitial fluid leads to a difference in pH of 0.013. The calculated difference in pressure between the blood compartment and the interstitial fluid counteracting the osmotic pressure difference is 3.2 kPa (24 mmHg). This pressure difference represents the mean hydrostatic pressure difference occurring there where the blood exchanges substance with the interstitial fluid, *i.e.* in the capillaries.

Loading and unloading of the IIE with CO_2 is attained by changing the pCO_2

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Table II. Calculated values of some quantities in the isolated internal environment at standard composition and conditions.

Quantity	Unit	Ery	Plasma	ISF	IIE
S_{O_2}		0.76*			
pCO_2	kPa	6.13*	6.13*	6.13*	6.13*
T	K	310*	310*	310*	310*
pH		7.184	7.379	7.392	
Volume	l	2.406	2.914	11.605	16.925
H_2O	l	1.727	2.740	11.260	15.727
Hemoglobin	$g \cdot l^{-1}$	332			
Protein	$g \cdot l^{-1}$		70.3	35.0	
ATP	$mmol \cdot kg^{-1}$	1.3			
2,3-DPG	$mmol \cdot kg^{-1}$	5.4			
Na^+	$mmol \cdot kg^{-1}$		164.5	159.6	
K^+	$mmol \cdot kg^{-1}$	167.4			
Cl^-	$mmol \cdot kg^{-1}$	75.2	117.7	121.3	
HCO_3^-	$mmol \cdot kg^{-1}$	18.1	28.4	29.3	
π_{coll}	kPa	105.6	3.5	1.3	
Δp_h	kPa	3.2	3.2	0*	

* Assumed values.
ISF = interstitial fluid.

in the IIE. In this way the amount of CO_2 in the body can be varied and the CO_2 equilibration curve of the IIE obtained (fig. 2). In table III the CO_2 contents of the three compartments are given, partitioned into the contributions of the different compounds to the standard composition at the standard state. In table IV concentrations related to 1.0 kg H_2O and 1.0 kg body mass are given. From table III it follows that in the blood of the IIE 122.9 mmol CO_2 (2.74 l STPD) is stored. This agrees with the value of 2.70 l reported by Cherniak and Longobardo (11). The CO_2 content of the IIE per kg body mass is 6.66 mmol. This value is difficult to compare with experimental values because these always refer to particular tissues or to the whole body. However, to compare the experimental values with the calculated one we can make the following estimation. The calculated concentration of HCO_3^- in the extracellular fluid (plasma + interstitial fluid) is 28.9 mmol $\cdot$ kg^{-1} H_2O, which is about three times the concentration in intracellular water in tissue cells of 9.6 mmol $\cdot$ kg^{-1} H_2O (2). Because the amount of intracellular water is about twice the amount of extracellular water, it contains $2 \times \frac{1}{3}$ times the extracellular amount of HCO_3^-

Table III. Total carbon dioxide content of the IIE at standard composition and conditions.

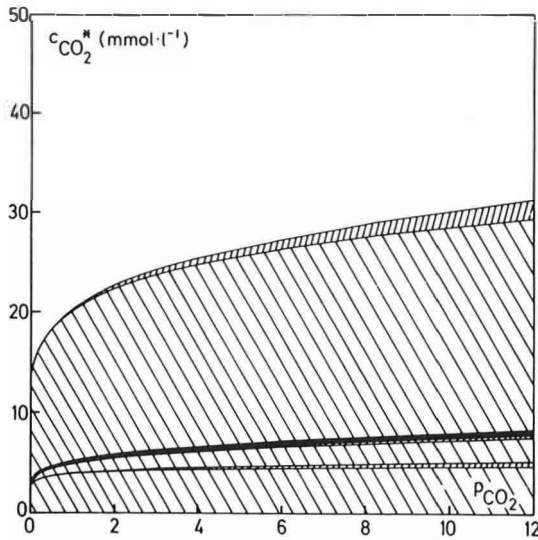| Quantity	Unit	Ery	Plasma	ISF	IIE
HCO_3^-	mmol	31.1	77.3	327.2	435.9
dissolved CO_2	mmol	2.9	4.1	16.3	23.3
carbamino CO_2	mmol	7.5			7.5
total CO_2	mmol	41.5	81.4	343.5	466.8

Fig. 2. Calculated concentrations of the various forms in which CO_2 is present in one liter of IIE as functions of pCO_2. From bottom to top: bicarbonate in plasma, dissolved CO_2 plus carbonic acid in plasma, bicarbonate in erythrocytes, dissolved CO_2 plus carbonic acid in erythrocytes, carba-mino-hemoglobin, bicarbonate in interstitial fluid, and dissolved CO_2 plus carbonic acid in interstitial fluid. The oxygen saturation of hemoglobin is 0.76.

which is $\frac{2}{3} \cdot 404.5 = 270$ mmol. Together with the total CO_2 of the internal environment of 466.5 mmol, this is 736.5 mmol fast exchanging CO_2 in the whole body, thus 10.5 mmol $\cdot$ kg^{-1} body mass. This value is well within the range of experimental results (Irving *et al.* (17) 10 mmol $\cdot$ kg^{-1}; Wallace and Hastings (27) 11 mmol $\cdot$ kg^{-1}; Fenn (14) 10 mmol $\cdot$ kg^{-1}; Albers *et al.* (2) 8.6 mmol $\cdot$ kg^{-1}).

To obtain a complete CO_2 equilibrium curve of the body, the amounts of CO_2 present at different values of pCO_2 must be known. Instead of these absolute values, which are difficult to measure, the slope of the equilibrium curve, approximated as a straight line, is measured. Reported values are for humans per kg body mass 0.48 ± 0.06 ml/kg$^{-1} \cdot$ torr^{-1} (0.16 ± 0.02 mmol $\cdot$ kg$^{-1} \cdot$ $\cdot$ kPa^{-1}) for equilibrations of short duration (1–8 min) (11). Values obtained

Table IV. Calculated values of the concentrations of bicarbonate and total CO_2 per kg water of the compartment concerned and per kg body mass.

Quantity	Unit	Ery	Plasma	ISF	IIE
HCO_3^-	mmol $\cdot$ kg^{-1} H_2O	18.1	28.4	29.3	27.7
total CO_2	mmol $\cdot$ kg^{-1} H_2O	24.0	29.9	30.8	29.9
HCO_3^-	mmol $\cdot$ kg^{-1} BM	0.44	1.10	4.67	6.22
total CO_2	mmol $\cdot$ kg^{-1} BM	0.59	1.16	4.91	6.66

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for longer equilibrations differ even more: 1.8 ± 0.4 ml·kg^{-1}·torr^{-1} (0.61 ± 0.14 mmol·kg^{-1}·kPa^{-1}) after equilibrations of 20–60 min duration (11). (One greatly deviating value has been omitted by us from the calculation of this mean value.) At the standard state the calculated slope of the CO_2 equilibration curve of the IIE is 0.24 mmol·kg^{-1}·kPa^{-1}, which is about 50% more than the value mentioned in the literature for short term equilibration. From the slope as calculated for the IIE by us it follows that for a breathholding man of 70 kg with a supposed rate of CO_2 production of 10 mmol/min, the pCO_2 would rise by 0.6 kPa/min (4.5 mmHg/min) if this CO_2 would be buffered in the IIE only. This agrees with the 5 mmHg/min mentioned by Cherniak and Longobardo (11). Of the amount of 0.24 mmol·kg^{-1}·kPa^{-1}, about 30% (0.075 mmol·kg^{-1}·kPa^{-1}) is buffered by the blood as part of the IIE. It is not justifiable to compare the value of 0.24 mmol·kg^{-1}·kPa^{-1} with the reported 0.61 mmol·kg^{-1}·kPa^{-1} for long term equilibration.

In our model only fast exchanging parts of the extracellular compartment are incorporated. Compounds like bone and tendon exchange matter only slowly, bone being the largest store of carbon dioxide compounds in the body. Cells contribute to the storing of CO_2 in an unknown way, and exchange of HCO_3^- in the kidney and the intestines makes that the experimental long term results cannot be compared with the calculated value, except for the conclusion that in the long run the combined renal, interstitial and cellular contribution to CO_2 "buffering" is large compared with that of the internal environment.

If in the model the amounts of all substances except CO_2 – in all its compounds – are kept fixed, a one-to-one relation exists between plasma pH and
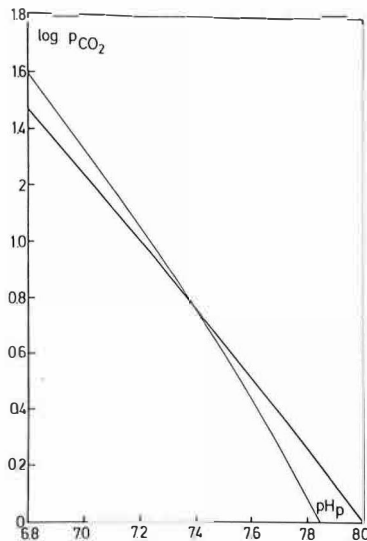

Fig. 3. Relations between log (pCO_2) and plasma pH as calculated for blood *in vitro* and in the isolated internal environment (thick line). In both the oxygen saturation is 0.76.

log ($p\mathrm{CO}_2$). This relation is given in fig. 3. The same relation for blood *in vitro* is also shown. The slope of the line for blood is $-1.64 \cdot \mathrm{pHu}^{-1}$, that of the IIE $-1.23 \mathrm{pHu}^{-1}$. The slope of the curve of blood *in vitro* as experimentally determined can be compared with the calculated one because the conditions are the same. For *in vivo* experiments the results can hardly be compared with the calculated curve because of the reasons already mentioned. Experimental values are $-1.60 \pm 0.07 \mathrm{pHu}^{-1}$ for blood *in vitro* and $-1.45 \pm 0.13 \mathrm{pHu}^{-1}$ for arterial blood *in vivo* [20]. As expected, the measured slope of the *in vivo* line is larger than that calculated for the IIE.

From the two preceeding relations the relation between the amount of CO_2 in the IIE and plasma pH follows. For the standard IIE this relation is given in fig. 4. It shows how the amounts of the various compounds in which CO_2 is present are related to plasma pH. From this it can be derived that at $\mathrm{pH}_p = 7.41$ the change in total CO_2 per kg body mass per unit plasma pH if caused by CO_2 titration, is -3.43 mmol.

The calculated change in total bicarbonate in the extracellular fluid is $-144.13 \mathrm{mmol} \cdot \mathrm{pHu}^{-1}$, which is $-2.06 \mathrm{mmol} \cdot \mathrm{pHu}^{-1}$ per kg body mass. This quantity was experimentally determined by Albers *et al.* [2] who found a value of $-4.0 \mathrm{mmol} \cdot \mathrm{pHu}^{-1} \cdot \mathrm{kg}^{-1}$.

The influence of the interstitial fluid on the capacity to buffer CO_2 is clear from the plasma bicarbonate-plasma pH diagram (fig. 5). This diagram shows
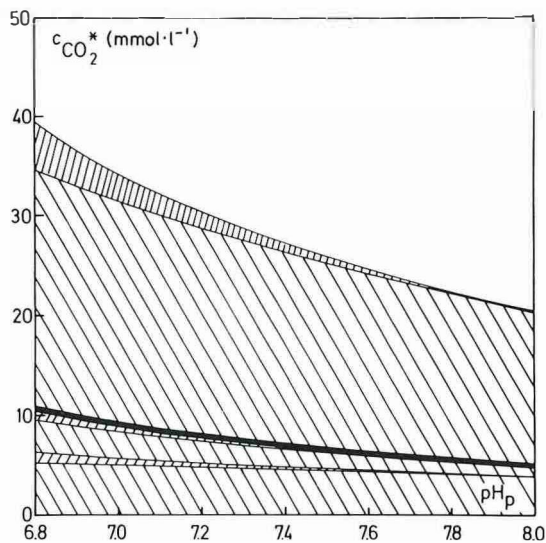
Fig. 4. Calculated concentrations of the various forms in which CO_2 is present in one liter of the IIE as functions of plasma pH. From bottom to top: bicarbonate in plasma, dissolved CO_2 plus carbonic acid in plasma, bicarbonate in erythrocytes, dissolved CO_2 plus carbonic acid in erythrocytes, carbamino-hemoglobin, bicarbonate in interstitial fluid, and dissolved CO_2 plus carbonic acid in interstitial fluid. The oxygen saturation of hemoglobin is 0.76.

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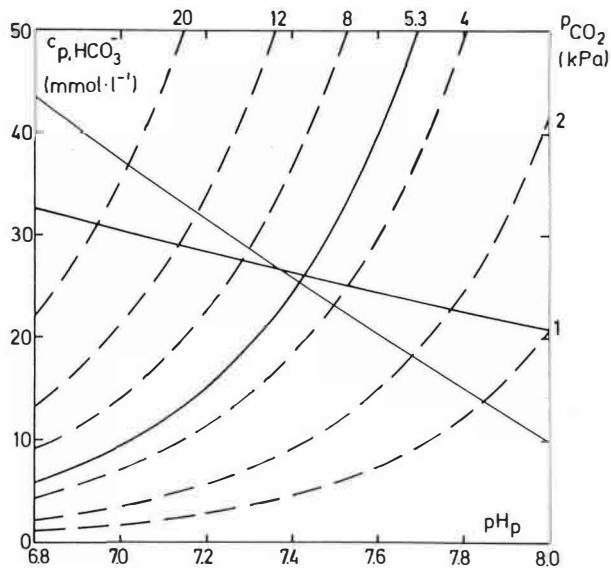Fig. 5. Calculated bufferline of the isolated internal environment (thick line), and of blood *in vitro* (thin line); $S_{O_2} = 0.76$. Several CO_2 isobars are given (hatched lines).

the curves calculated for blood *in vitro* and for blood in exchange with interstitial fluid. The calculated slope of the latter is -9.7 mmol·1^{-1}·pHu^{-1} while that of the former is -28.3 mmol·1^{-1}·pHu^{-1}. In table V values are given of the slope of the bufferline *in vivo* experimentally determined by means of whole body titration with CO_2 by various investigators. It must be realized that the *in vivo* bufferline is not only due to the buffering by the blood and the interstitial fluid but also represents contributions from the tissue cells, the kidneys and the intestines.

Fig. 6 shows the effect of CO_2 loading and unloading on the Donnan ratio. The thick line is the Donnan ratio for Cl^-, HCO_3^- and H^+ between plasma and red cells when blood exchanges ions and water with the interstitial fluid. The thin line is that calculated for blood *in vitro*. The influence of the redistribution of ions through the dilution with the interstitial fluid is also demonstrated in the changes in osmolality with pH (fig. 7). The osmotic changes in blood *in vitro* are substantially larger than those in blood in exchange with the interstitial fluid. This is also reflected in the changes in hematocrit and the volumes with pH as given in figs. 8 and 9.

DISCUSSION

The results of measurements concerning the change in acid-base status with changes in pCO_2 are represented in many forms. Frequently used variables are, first pH or a_{H^+}, second pCO_2 or log (pCO_2), and third c_{p,HCO_3^-}. This makes it

Table V. Experimental values of the slope of the CO_2 bufferline *in vivo*.

| HUMANS	slope $mmol \cdot l^{-1} \cdot pHu^{-1}$ (absolute value)	equilibration time (min)	number of individuals resp. dogs	circumstances
Böning and Heinrich (6)	8.3	15	10	exogenous hypercapnia
Böning *et al.* (7)	13	30	33	exogenous hypercapnia
	12	40	18	voluntary hypocapnia
Bracket *et al.* (8)	9.7	40–90	7	exogenous hypercapnia
Bunker (10)	17.6	60		
Ichiyanagi *et al.* (16)	21	15	8	exogenous hypercapnia and voluntary hypocapnia
	15	15	10	anesthesia, endogenous hypercapnia
	15	60	10	anesthesia, exogenous hypercapnia
	27	20	12	anesthesia, hypocapnia
Prys-Roberts *et al.* (20)	21 ± 6	40–60		anesthesia, exogenous hypercapnia, hypocapnia
DOGS				
Albers *et al.* (2)	23	30	13	anesthesia, hyper- and hypocapnia; kidneys ligated
Brown and Clancy (9)	11.8	15	6	anesthesia, hypercapnia
Brown and Michel*	9.3			venous blood
	12.2			arterial blood
Cohen *et al.* (12)	19	60	21	awake, exogenous
Garcia *et al.* (15)	12	15	6	anesthesia endogenous
	5	120		hypercapnia
	8	10	6	anesthesia, hypocapnia
	11	120		

* as quoted by Garcia *et al.* (15).
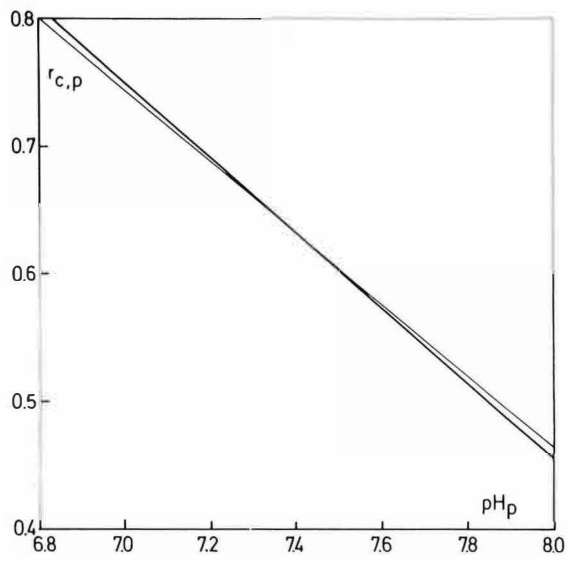
Fig. 6. Relation between plasma pH and the Donnan ratio erythrocyte-plasma as calculated for the isolated internal environment (thick line), and for blood *in vitro* (thin line); $S_{O_2} = 0.76$.
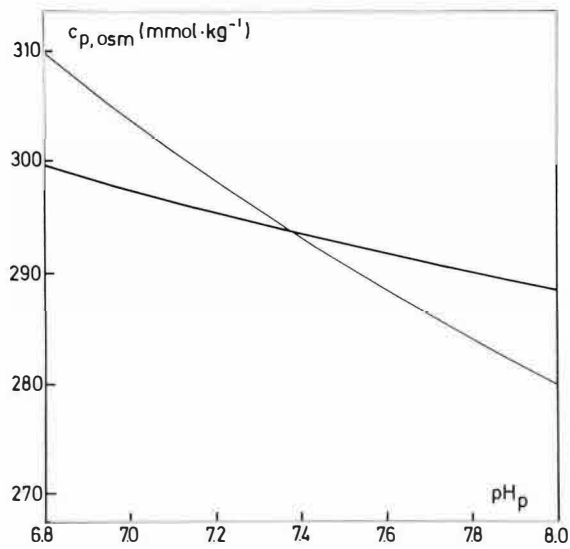

Fig. 7. Relation between the real osmolality of plasma and plasma pH as calculated for the isolated internal environment (thick line) and for blood *in vitro* (thin line); $S_{O_2} = 0.76$. In both cases 10 mmol/kg is added to the calculated osmolality to account for the osmotic contribution of glucose and urea.
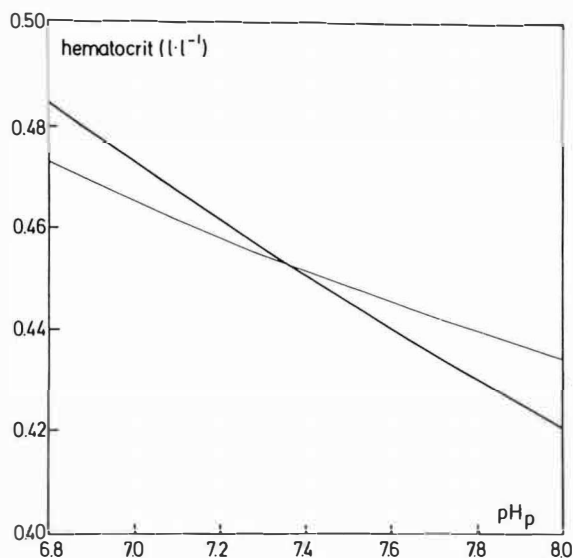
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Fig. 8. Changes in hematocrit versus plasma pH under CO_2 titration in the isolated internal environment (thick line) and in blood *in vitro* (thin line); $S_{O_2} = 0.76$.

difficult to compare results, because when a relation between two quantities is represented as linear, this linearity is not preserved after transformation to another set of variables. As far as possible, available results have been transformed to the $c_{p,\mathrm{HCO_3^-}}/pH_p$ diagram to make any comparison possible. An exception to this is the log $(pCO_2)/pH_p$ relation shown in fig. 3. As stated earlier, the slope of the $c_{p,\mathrm{HCO_3^-}}/pH_p$ bufferline *in vivo* in the steady state is the consequence of buffering by the blood, the interstitial fluid, the cells, and eventually of the action of the kidneys and the intestines. Thus the experimental results will probably differ from our calculated values.

From 1932 onwards, when Shaw and Messer (26) discussed the transfer of bicarbonate between the blood and the tissues, the difference in slope between blood *in vitro* and blood *in vivo* has been the subject of much debate (10). A simple way to account for the effect of the interstitial fluid on the slope of the bufferline is to assume that the blood is diluted with the interstitial fluid. This was put forward by Armstrong *et al.* (3) who used the resultant hematocrit in the calculations with the Singer-Hastings nomogram or the log $(pCO_2)/pH_p$ diagram. They showed that the outcomes were in agreement with experimental values. This line of reasoning was followed by others in their constructions of models of the isolated internal environment: Roos and Thomas (23), Dell and Winters (13) and Böning (5). In these studies values of buffer capacities are assigned to compartments without a detailed description of the various processes such as water, chloride and bicarbonate exchange. In this way a formula for the buffer capacity of the internal environment is derived in which the
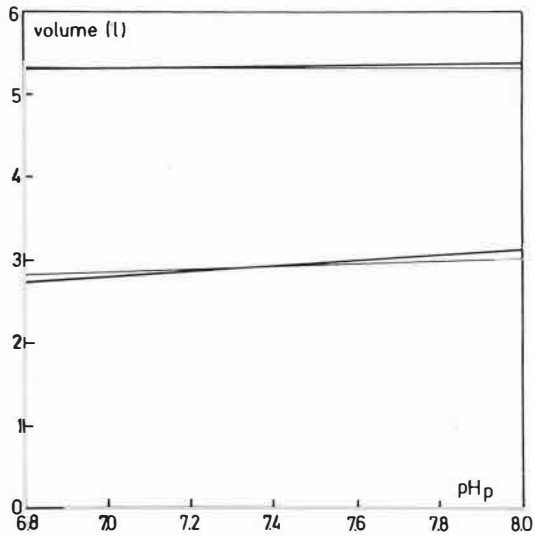
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Fig. 9. Changes in plasma volume (lower line) and in blood volume (upper line) versus plasma pH under CO_2 titration in the isolated internal environment (thick lines) and in blood *in vitro* (thin lines); $S_{O_2} = 0.76$.

quantities initially used appear. The influence of these quantities upon the buffer value can be assessed. Depending on the values of quantities used, the calculated values of the slope of the CO_2 bufferline differ widely in these studies. Roos and Thomas (23) arrive at a value of -10.1 mmol·l^{-1}·pHu^{-1}, in agreement with the value of -9.7 mmol·l^{-1}·pHu^{-1} calculated by us. When in the model of Dell and Winters (13) our values of the blood volume and the
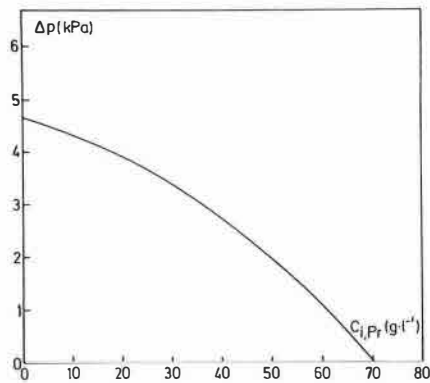

Fig. 10. Hydrostatic pressure difference between the blood compartment and the interstitial fluid compartment necessary to maintain constancy of the volumes of these compartments with various protein concentrations in the interstitial fluid; $S_{O_2} = 0.76$.

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amount of interstitial fluid are substituted, a slope of -18 mmol$\cdot$l$^{-1}\cdot$pHu^{-1} results, which is about twice the value calculated by us. The value that follows from the study of Böning (5) is about -16 mmol$\cdot$l$^{-1}\cdot$pHu^{-1}.

When $p\mathrm{CO}_2$ differs from normal, it is difficult to define a steady state, because the rate of exchange of the interstitial compartments with plasma differs widely. This makes it the more difficult to determine whether a steady state has been attained. It may be doubted whether in all of the reported experiments a real steady state was established. Furthermore, Roos and Thomas (23) have shown that changes in cardiac output lead to a variation in the slope of the CO_2 bufferline of arterial blood.

The model offers the possibility to study the effects of dilution by the interstitial fluid and the consequences of the addition, withdrawal and redistribution of CO_2, O_2, ions, protein and water. As an example a calculation was made in order to determine the hydrostatic pressure difference between capillary lumen and interstitium needed to maintain the partitioning of water between plasma and interstitium at the chosen proportion with various protein concentrations in the interstitial fluid. The outcome is given in fig. 10. It follows from this figure that for an ultrafiltrate from plasma with no protein content, the corresponding hydrostatic pressure difference across the capillary wall would be at least 4.7 kPa (35 mmHg).

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CHAPTER VI

CONCLUDING REMARKS

CONCLUDING REMARKS

The development of a mathematical model is to some extent comparable with the growth of a scientific theory. On the one hand the comparison of calculated results with experimental values gives an impression of the quality of the model, on the other hand the model provides new results which may lead to new experiments. The better the known results comply with reality, the more reliable new results become.

The results presented in the preceding chapters are to a large extent in agreement with experimental findings. However, there was one assumption necessary that is not in accordance with experimental results. This assumption concerns the osmotic behaviour of hemoglobin in solution. In the second chapter, the osmotic coefficient of hemoglobin was assumed to be equal to that of plasma protein. This value is larger than the highest experimental value, that of McConaghey and Maizels (5) (figure 12, chapter 2).

Recent determinations of the osmotic coefficient of haemoglobin by Wittmann and Gros (8) have confirmed the values of McConaghey and Maizels. However, analysis of the relations among variations in human red cell volume, density, membrane area, hemoglobin content and cation content by Svetina (7) shows much more influence of hemoglobin and less influence of cations on the osmotic behaviour of the red cell than is expected from the generally assumed osmotic behaviour of red cells. Because in the model all the variables are inter-related, it is not possible to change the osmotic properties of hemoglobin without influencing other characteristic quantities. This discrepancy may be removed by introducing refinements in the model. The hemoglobin solution in the red cell compartment was assumed to fill the whole of the red cell volume, regardless of other voluminous constituents like, for example, the cell membrane. In the model the volume of the latter is reckoned in the volume of hemoglobin by assuming that 1 g of hemoglobin occupies 0.85 mL instead of 0.75 mL. The extra volume of 15 mL in 1 L blood is probably somewhat more than can be attributed to membranes 10 nm thick. Such a membrane would occupy about 8 mL in blood with 150 g/L hemoglobin. Taking this volume apart leads to a slight rise of the hemoglobin concentration in the solution filling the red cell compartment. Furthermore, the hematocrit measured in blood includes more than only the red cell volume fraction because of plasma trapping. This means that the actual intracellular hemoglobin concentration comes out higher than assumed in the model. Thus, instead of the higher osmotic coefficient it appears to be more justified to use a higher than supposed hemoglobin concentration in the red cell, i.e. about 340-350 g/L. This shift in the assumptions indeed leads to more or less the same results as with the original assumption of the higher osmotic coefficient while using a proven relation for the osmotic

pressure of hemoglobin.

The model presented is suited to calculate equilibrium values of quantities but not the course of these values during exchange of substances. Nevertheless, when a process can be split up into a succession of equilibria it is possible to quantitate the effects of certain exchanges by looking at differences between these equilibrium states. The model of blood in an earlier version has been used in this way to estimate the influence of the availability of carbonic anhydrase to blood plasma on the gas exchange in the lungs (9).

Observations of postcapillary changes in blood pH (1, 4) and on pH changes in the aorta (6) strongly indicate that there is no equilibrium in the blood when it leaves the pulmonary capillaries. Two processes are slow enough to cause this: firstly, the uncatalyzed dehydration and hydration of CO_2 and, secondly, the HCO_3^-/Cl^- exchange across the red cell membrane. Until recently, it was supposed that all reactions of the gas exchange in the lungs reached equilibrium at the end of the pulmonary capillary and that the rapid CO_2 exchange was due to carbonic anhydrase catalytic activity inside the erythrocytes. The HCO_3^-/Cl^- exchange was supposed not to be a limiting factor, so that the plasma HCO_3^- could be converted into CO_2 inside the erythrocyte as well. This picture has changed since the observation that the blood pH in arterial blood changes during the passage through the aorta (1, 4, 6) and the discovery of carbonic anhydrase at the endothelium of the pulmonary capillaries (2, 3). The former has been interpreted as indicating that physico-chemical equilibria have not yet settled in arterial blood in the aorta.

In order to study the influence of the availability of carbonic anhydrase to plasma, it was assumed that the uncatalyzed hydration and dehydration of CO_2 and the HCO_3^-/Cl^- exchange proceed so slowly that the progress of these processes during the gas exchange in the lungs is negligible in comparison with their progress afterwards. This makes it possible to add an intermediate equilibrium stage. Venous blood entering the lungs is supposed to be in equilibrium. The gas exchange takes place before the intermediate stage, and the other equilibrations are assumed to occur between the intermediate stage and the final equilibrium stage. The change in pH between these stages represents the maximum change that can occur in arterial blood in the aorta. This value should therefore be compared with experimental results.

In the model the presence of carbonic anhydrase is taken into account by the way plasma was assumed to equilibrate with alveolar gas. With carbonic anhydrase available, both plasma and erythrocyte content reach complete equilibrium with the gas phase, but separately. When no carbonic anhydrase is available to plasma, only the red cell content was assumed to reach complete equilibrium with the alveolar gas, plasma being assumed to keep its venous composition with regard to HCO_3^- and to equilibrate with regard to dissolved

CO_2. In both cases, pH in plasma was calculated. After the intermediate stage the exchange between plasma and red cells was assumed to take place in closed system conditions. For the final stage both pH and pCO_2 were calculated. There are, of course, two final states corresponding to the two types of gas exchange assumed. The results obtained with this approach were in line with the experimental results (9). In this way the extreme values of exchange processes in certain conditions can be calculated.

The model described in chapter 5 was used to study the influence of the protein concentration in plasma and the interstitial fluid on the whole body buffer line for CO_2. It turned out (10) that these quantities are just as important as hemoglobin concentration, although, generally, more emphasis is put on the latter.

The results discussed in the previous chapters show that many of the physico-chemical properties of blood and interstitial fluid in relation to blood gas and acid-base equilibria can be derived from the properties of the constituents. The results presented are in no way exhaustive, nor can the model in its present state be considered an end point. Besides the problem of the osmotic pressure there are other points that deserve attention in order to improve and extend the model. A desired extension is the introduction of 2,3-DPG binding to hemoglobin. Although rather complex to describe, it should be possible to incorporate this binding into the model as well. Eventually, this model of blood can be used as an educational tool for medical students.

It is our intention to apply the method to calculate exchanges of O_2 and CO_2 from differences between equilibrium states as presented above for the estimation of carbonic anhydrase influence in the lung capillaries also to the gas exchange in the tissues. To simulate the O_2 and CO_2 exchanges in the body in steady state, the cardiac output will be introduced into the model. Assuming that equilibria of some kind will be attained both in the lungs and in the tissues, steady state exchanges can be calculated as differences in total CO_2 and total O_2 contents between arterial and venous blood multiplied by the cardiac output. The equilibria in the tissues can be calculated with the model of the isolated internal environment. The pulmonary gas exchange will be calculated with a simple steady state model of the ventilation.

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SAMENVATTING

Het onderwerp

Een levend wezen als de mens bestaat, schematisch gezien, uit een zeer groot aantal levende cellen die zijn ingebed in een vloeistofhoudende tussenstof die niet "levend" is, maar die door de cellen wordt onderhouden. Hiervoor en voor hun eigen leven moeten de cellen kunnen beschikken over energie. De energie wordt verkregen door beheerste verbranding van voeding. Daartoe heeft de cel zuurstof nodig en ook moet de bij de verbranding vrijkomende kooldioxide worden afgevoerd. De zuurstof komt uit de buitenlucht en het kooldioxide moet daarin worden geloosd. Voor deze uitwisseling zorgen de longen. Voor het verdere transport zorgt het rondstromende bloed. Voor deze functie bevat het bloed speciale cellen, de rode bloedcellen, die daartoe vol zitten met een speciaal eiwit, het hemoglobine. Dit eiwit geeft het bloed zijn karakteristieke, rode kleur. Het transport van zuurstof gebeurt voor meer dan 90% door binding aan het hemoglobine. Ter plaatse van de verbruikers, de lichaamscellen, wordt de zuurstof losgelaten. Dit loslaten wordt bevorderd door de omstandigheden ter plaatse: een zuurdere omgeving door het koolzuur en een temperatuur en koolzuurgasspanning die hoger is dan in het toestromende bloed. De gevoeligheid van het hemoglobine voor deze faktoren is een zeer functionele bijzonderheid van dit eiwit. Het hemoglobine wordt niet direkt geconfronteerd met de veranderde omstandigheden doordat het in de rode bloedcellen opgesloten zit. Het inwendige van de cel verandert wel met de omgeving mee, maar in een afgezwakte vorm. Doordat er tussen de ruimte om de lichaamscellen en het bloed nog de scheiding door de bloedvaatwand is, werken de veranderingen rondom de cellen ook in gemodificeerde vorm door op het bloed. Het verband tussen veranderingen rondom de cellen, in de interstitiële vloeistof en de veranderingen in bloed is daardoor gecompliceerd.

Het proefschrift

De bovenbeschreven wisselwerkingen tussen zuurstof en kooldioxide en hun invloed op de verdeling van water en elektrolyten tussen bloedplasma en de rode cellen zijn uitgebreid bestudeerd. Experimenten daarover kunnen niet het gehele samenspel tegelijk betreffen omdat het ondoenlijk is alle variabelen te meten. Om toch inzicht te krijgen in het totale systeem is al snel geprobeerd de samenhang tussen de grootheden in grafieken en vergelijkingen te vangen. Eertijds was het gebruik van nomogrammen de enige methode om deze vergelijkingen met verschillende parameters op te lossen. In 1928 volgde Henderson deze methode in zijn boek "Blood". Toch is het zelfs hiermee zeer bewerkelijk en weinig nauwkeurig om samenhangen vorm te geven. Met de

komst van de rekenmachine kwam een nieuwe mogelijkheid om dit te doen, beschikbaar.

De eerste modellen waren gebaseerd op enige vereenvoudigende veronderstellingen. Zo'n model was ook in ons laboratorium in gebruik. Uitgaande daarvan is een uitgebreider en verfijnder model ontwikkeld dat in dit proefschrift wordt beschreven. Verfijningen en uitbreidingen betreffen het invoeren van osmotische en donnan evenwichten, het invoeren van adenosinetrifosfaat (ATP) en 2,3-difosfoglyceraat (2,3-DPG) als buffers in de rode cellen, en het uitbreiden van de in rekening gebrachte eigenschappen van hemoglobine. Daarnaast is ook het evenwicht van bloed met de interstitiële vloeistof onderwerp van studie. Een beperking is, dat in het model alleen de bufferwerking van het 2,3-DPG in aanmerking is genomen en de invloed ervan op het hemoglobine slechts impliciet aanwezig is. Voor het overige leiden de modellen in een veelheid van condities tot berekende waarden die goed in overeenstemming zijn met bekende experimentele resultaten. Zij kunnen daarom als een redelijke goede representatie van dit stukje werkelijkheid worden beschouwd.