

1 **A mechanistic physicochemical model of carbon dioxide transport in blood**

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16

17 **Abstract**

18 A number of mathematical models have been produced that, given the PCO_2 and PO_2
19 of blood, will calculate the total concentrations for CO_2 and O_2 in blood. However, all
20 these models contain at least some empirical features, and thus do not represent all of
21 the underlying physicochemical processes in an entirely mechanistic manner. The aim
22 of this study was to develop a physicochemical model of CO_2 carriage by the blood to
23 determine whether our understanding of the physical chemistry of the major chemical
24 components of blood together with their interactions is sufficiently strong to predict
25 the physiological properties of CO_2 carriage by whole blood. Standard values are used
26 for the ionic composition of the blood, the plasma albumin concentration and the
27 haemoglobin concentration. All K_m values required for the model are taken from the
28 literature. The distribution of bicarbonate, chloride and H^+ ions across the red cell
29 membrane follows that of a Gibbs-Donnan equilibrium. The system of equations that
30 results is solved numerically using constraints for mass balance and electro-neutrality.
31 The model reproduces the phenomena associated with CO_2 carriage, including the
32 magnitude of the Haldane effect, very well. The structural nature of the model allows
33 various hypothetical scenarios to be explored. Here we examine the effects of: i)
34 removing the ability of haemoglobin to form carbamino compounds; ii) allowing a
35 degree of Cl^- binding to deoxygenated haemoglobin; and iii) removing the chloride
36 (Hamburger) shift. The insights gained could not have been obtained from empirical
37 models.

38

39 **New & Noteworthy**

40 This study is the first to incorporate a mechanistic model of chloride-bicarbonate
41 exchange between the erythrocyte and plasma into a full physicochemical model of
42 the carriage of carbon dioxide in blood. The mechanistic nature of the model allowed
43 a theoretical study of the quantitative significance for carbon dioxide transport of
44 carbamino compound formation; the putative binding of chloride to deoxygenated
45 haemoglobin and the chloride (Hamburger) shift.

46

47 **Keywords:**

48 Blood, mathematical model, Haldane effect, gas transport, carbon dioxide
49 dissociation, carbon dioxide carriage

50

51 **Glossary**

52

Symbol	Description	Units
ISID	Impermeant strong ion difference	meq. l ⁻¹
K_x	Equilibrium constant	variable
P	(Partial) pressure	mmHg
PSID	Permeant strong ion difference	meq. l ⁻¹
R	Respiratory quotient	
R_0	Universal gas constant	J/kmol.K
S_{O_2}	Oxygen saturation	
SID	Net strong ion difference	meq. l ⁻¹
T	Temperature	K
c	Dissolved concentration (of a gas)	mmol.l ⁻¹
f	Fraction	
pK_x	$-\log_{10}(\text{Equilibrium constant})$	n/a
q	Charge concentration	meq. l ⁻¹
t	Total content (of a gas)	ml _{STPD} . dl ⁻¹
z	Mean valence	meq. mmol ⁻¹
ΔH°	Enthalpy of conformation	kJ. mol ⁻¹
α_x	Henry's law coefficient of solubility	mmol. Pa ⁻¹
[Alb]	Albumin concentration	mmol. l ⁻¹
[Ca ²⁺]	Calcium concentration	mmol. l ⁻¹
[Cl ⁻]	Chloride concentration	mmol. l ⁻¹
[CO ₃ ²⁻]	Carbonate ion concentration	mmol. l ⁻¹
[H ⁺]	Hydrogen ion activity	mmol. l ⁻¹
[Hb]	Haemoglobin concentration	mmol. l ⁻¹
[HCO ₃ ⁻]	Bicarbonate ion concentration	mmol. l ⁻¹
[K ⁺]	Potassium concentration	mmol. l ⁻¹
[SO ₄ ²⁻]	Sulphate concentration	mmol. l ⁻¹
[Lac ⁻]	Lactate concentration	mmol. l ⁻¹
[Mg ²⁺]	Magnesium concentration	mmol. l ⁻¹
[Na ⁺]	Sodium concentration	mmol. l ⁻¹
[OH ⁻]	Hydroxide ion activity	mmol. l ⁻¹
[OrgPhos]	Organic phosphate concentration	mmol. l ⁻¹
[Phos]	Phosphate concentration	mmol. l ⁻¹
Subscripts		
Alb	Albumin	
AlbC	Carboxy terminus of albumin	
AlbHis	Histidine residue of albumin	
AlbZ	Amino terminus of albumin	

Asp/Glu	Aspartic or glutamic acid
Cys	Cystine
CO ₂	Carbon dioxide
DPG	2,3-disphosphoglycerate
DPG2a	Second dissociation constant for the first of the two phosphate groups of 2,3-disphosphoglycerate
DPG2b	Second dissociation constant for the second of the two phosphate groups of 2,3-disphosphoglycerate
HbC	Carboxy terminus of haemoglobin
HbCO	Carboxy-haemoglobin
Hbdeoxy	Deoxygenated haemoglobin
HbF	Fetal haemoglobin
HbHis	Histidine residue of haemoglobin
Hbmet	Methaemoglobin
HbO ₂	Oxygenated haemoglobin
HbZ	Amino terminus of haemoglobin
Lys/Arg	Lysine or arginine
NH ₃ ⁺	Ionised amino terminus
NHCOO ⁻	Ionised carboxy terminus
O ₂	Oxygen
P1/P2/P3	First, second, and third phosphate ionisation
R	Relaxed state of haemoglobin
T	Tense state of haemoglobin
Tyr	Tyrosine
H ₂ O	Water
<i>b</i>	Whole blood
<i>c</i>	Cell (erythrocyte)
<i>i</i>	The <i>i</i> th component in a summation
<i>p</i>	Plasma
<i>v̄</i>	Mixed venous
α	Alpha chain of haemoglobin
β	Beta chain of haemoglobin

54 **Introduction**

55 The carriage of CO₂ by the blood is complex. It is present in a physically dissolved
56 state. It also undergoes reversible reactions to form carbamino compounds with
57 haemoglobin, and to form bicarbonate. In these reactions protons are generated. They
58 are buffered principally by haemoglobin, and to a lesser degree by albumin and
59 inorganic/organic phosphate. While the process of proton buffering is mainly
60 intracellular, most of the bicarbonate is in the plasma, and so chloride/bicarbonate
61 exchange occurs across the red cell membrane (17). Finally, haemoglobin exists in
62 both tense (T, deoxygenated) and relaxed (R, oxygenated) allosteric forms, which
63 have different affinities for CO₂ binding, and different pKs for the histidine residues
64 that buffer protons. It is this last feature that underlies the Haldane effect (9), where
65 deoxygenated blood holds a greater amount of CO₂ than does oxygenated blood at the
66 same partial pressure of CO₂.

67 Fundamental to understanding the carriage of CO₂ by blood is the notion of a
68 dissociation curve which gives the relationship between the PCO₂ of the gas or tissue
69 to which the blood is exposed and its overall CO₂ content. Because of the Haldane
70 effect, the dissociation curve depends on the relative proportions of haemoglobin in
71 each allosteric form. Thus, a core problem is to determine, for any given pair of PCO₂
72 and PO₂ values, what are the total blood contents (including all reversibly bound
73 forms) for CO₂ and O₂.

74 This particular problem has given rise to a number of empirical relationships, initially
75 in the form of nomograms (11, 18-20, 49, 53), and later as empirical mathematical
76 models (27, 31, 33, 35). However, these models are simply descriptive. None sets out
77 to model the underlying physical chemistry of each of the molecular species that plays

a significant role in gas carriage, and consequently build up the overall gas carrying properties of blood from first principles. Consequently, none of these models can be used to study the importance of certain aspects of the physical chemistry to gas carriage by modifying the property in the model to determine the effect.

There are other models, generally more recent, that are more mechanistic by nature. Some have been directed towards modelling the dynamics of the reactions in blood (7, 8, 22), while others have been directed more towards modelling the acid-base status of blood rather than gas carriage (45, 46, 62). Wooten's model (62) used individual physicochemical constants for the buffering of protons by haemoglobin, but the model was restricted to completely oxygenated blood. The model of Rees *et al.* (45, 46) did incorporate a model for varying haemoglobin saturation, but the actual model for buffering of protons by haemoglobin was phenomenological in nature and another significant limitation was the absence of a model for ionic exchange across the red cell membrane as PCO_2 and PO_2 were varied.

In this study, we set out to build a mechanistic, physicochemical model of CO_2 carriage by the blood. It uses physicochemical constants published for albumin (13) and for both the T and R states of haemoglobin (12, 32). These constants are coupled with the requirements for mass balance, for electro-neutrality and for the need for permeant ions to distribute across the red cell membrane in accordance with a Gibbs-Donnan equilibrium system. The main question we set out to address was whether such a model was sufficiently complete that it could reproduce the physiological properties of whole blood. Effectively, the physicochemical constants are the building blocks for the model and the physiological properties form the validation data set for the model.

Following model validation, we used the mechanistic nature of the model to explore the importance to CO₂ transport of the amino termini of the globin chains by successively removing from the model, first the ability of these to form carbamino compounds, and secondly their ability either to form carbamino compounds or buffer protons. We explored the importance of chloride binding to deoxygenated haemoglobin by introducing this property to the model. Finally we examined the role of the chloride (Hamburger) shift on CO₂ transport. Such “what if” experiments in general are only possible with a model that reflects the underlying physicochemical principles and cannot be undertaken using empirical models.

Methods

The core problem to solve is, given the PCO₂ and PO₂ of blood (which we assume to be the same both in plasma and inside the red blood cell), calculate the total content of CO₂ and O₂ in the blood. This content is to include both the gas that is reversibly reacted with the blood as well as that physically dissolved in blood.

For carbon dioxide, which is dissolved and also carried as bicarbonate and carbonate in both plasma and erythrocytic fluid, and also bound to the amino termini of haemoglobin chains, the expression for total carbon dioxide content, t_{CO_2} , is:

$$t_{\text{CO}_2} = f_p(\alpha_{\text{CO}_2,p}\text{PCO}_2 + [\text{HCO}_3^-]_p + [\text{CO}_3^{2-}]_p) + f_c(\alpha_{\text{CO}_2,c}\text{PCO}_2 + [\text{HCO}_3^-]_c + [\text{CO}_3^{2-}]_c) \\ + (f_{\text{HbR,NHCOO}^-} + f_{\text{HbT,NHCOO}^-}) \times 4[\text{Hb}]_b \quad (1)$$

where f_p and f_c are the volume fractions of plasma and intracellular fluid respectively, $\alpha_{\text{CO}_2,p}$ and $\alpha_{\text{CO}_2,c}$ are the coefficients of solubility for CO₂ in plasma and intracellular fluid respectively, $f_{\text{HbR,NHCOO}^-}$ and $f_{\text{HbT,NHCOO}^-}$ are the fractions of haemoglobin in the relaxed carbamino- and tense carbamino- states respectively (to give the overall

124 fraction of Hb reacted to form carbamino compounds), and $[\text{Hb}]_b$ is the concentration
125 of haemoglobin tetramers in whole blood.

126 For oxygen, which is both physically dissolved in the fluid of the blood and bound to
127 haemoglobin, the expression for total content, t_{O_2} , is:

$$t_{\text{O}_2} = \alpha_{\text{O}_2,b} P_{\text{O}_2} + f_{\text{HbO}_2} \times 4[\text{Hb}]_b, \quad (2)$$

128 where $\alpha_{\text{O}_2,b}$ is the coefficient of solubility for oxygen in whole blood, and f_{HbO_2} is the
129 fraction of haemoglobin that is oxygenated.

130 For both *Eq. 1* and 2, each quantity on the right-hand side is one of four distinct
131 entities. It is either: a) an input variable to the model (PCO_2 and PO_2); or b) a known
132 physicochemical constant (the coefficients of solubility: $\alpha_{\text{CO}_2,p}$, $\alpha_{\text{CO}_2,c}$, and $\alpha_{\text{O}_2,b}$, see
133 Table 1); or c) a starting parameter for blood (f_p , f_c , and $[\text{Hb}]_b$) which does not change
134 with PCO_2 or PO_2 – these are described in the section “*Setting the Blood Parameters*”,
135 below; or d) a chemical moiety ($[\text{HCO}_3^-]_p$, $[\text{CO}_3^{2-}]_p$, $[\text{HCO}_3^-]_c$, $[\text{CO}_3^{2-}]_c$, $f_{\text{HbR,NHCOO}^-}$,
136 $f_{\text{HbT,NHCOO}^-}$, and f_{HbO_2}) that varies with the PCO_2 and PO_2 of blood. It is the estimation
137 of the variables in this last category that forms our principal problem.

138 The overall approach used to construct the model is as follows. First, the law of mass
139 action is used to express each of the chemical moieties that varies with PCO_2 and PO_2
140 (listed under d) above) in terms of: a) the input variables (PCO_2 and PO_2); b) the
141 physicochemical constants (Table 1); c) the parameters of blood that do not vary with
142 PCO_2 and PO_2 ; and d) the plasma and/or intracellular $[\text{H}^+]$ concentrations. These
143 expressions are grouped under the following section entitled “*Applying the Law of*
144 *Mass Action*” below. This section also contains similar expressions for other chemical
145 moieties that will be required later in the model.

146 The second step in specifying the model is to use the physical requirement for charge
 147 neutrality together with the Gibbs-Donnan equilibrium condition for ionic
 148 distributions across the red cell membrane to solve for the plasma and intracellular
 149 $[H^+]$. This step is detailed in the section “*Physical Constraints for Plasma and*
 150 *Intracellular $[H^+]$* ” below.

151 Finally, there is a section to describe how the initial parameters for blood are set
 152 (*Setting the Blood Parameters*), and a section concerning the method used to solve the
 153 equations of the model (*Solution Method*).

154

155 *Applying the Law of Mass Action*

156 Here we specify the equations resulting from the law of mass action for each of the
 157 weakly ionised species considered in the model. These species are water, carbonic
 158 acid, inorganic and general organic phosphate, 2, 3-diphosphoglycerate (2,3-DPG),
 159 albumin and haemoglobin. The symbol K with the relevant suffix is used for all
 160 equilibrium constants, and values for these are listed in Table 1.

161 For water, we may write:

$$[OH^-] = \frac{K_{H_2O}}{[H^+]}, \quad (3)$$

162 and for the carbonic acid system we may write:

$$[HCO_3^-] = \frac{K_{CO_2,1} \alpha_{CO_2} P_{CO_2}}{[H^+]}, \quad (4)$$

$$[CO_3^{2-}] = \frac{K_{CO_2,2} K_{CO_2,1} \alpha_{CO_2} P_{CO_2}}{[H^+]^2}. \quad (5)$$

163 For the other weakly ionised species the total concentrations are taken as fixed
 164 (Table 1), which means it is necessary only to determine the mean valence (charge per
 165 mole).

166 Phosphate is present in inorganic and organic forms, both within the plasma and the
 167 erythrocyte. For all phosphates except erythrocytic 2,3-DPG, this is modelled as a
 168 single phosphate group which ionises successively, and where the mean valence is
 169 calculated from:

$$z_{\text{Phos}} = \frac{(K_{\text{P1}}[\text{H}^+]^2 + 2K_{\text{P2}}[\text{H}^+] + 3K_{\text{P1}}K_{\text{P2}}K_{\text{P3}})}{([\text{H}^+]^3 + K_{\text{P1}}[\text{H}^+]^2 + K_{\text{P1}}K_{\text{P2}}[\text{H}^+] + K_{\text{P1}}K_{\text{P2}}K_{\text{P3}})} \quad (6)$$

170 Erythrocytic 2,3-DPG is quantitatively the most important phosphate, and the
 171 ionisation states of the two phosphate groups are not independent. Here it is modelled
 172 as two phosphate groups that share the same K_{P1} and K_{P3} as above, but have two
 173 different values for $K_{\text{P2}} - K_{\text{DPG2a}}$ and K_{DPG2b} – as reported by Hobish and Powers (23),
 174 thus:

$$z_{\text{DPG}} = \frac{(K_{\text{P1}}[\text{H}^+]^2 + 2K_{\text{DPG2a}}[\text{H}^+] + 3K_{\text{P1}}K_{\text{DPG2a}}K_{\text{P3}})}{([\text{H}^+]^3 + K_{\text{P1}}[\text{H}^+]^2 + K_{\text{P1}}K_{\text{DPG2a}}[\text{H}^+] + K_{\text{P1}}K_{\text{DPG2a}}K_{\text{P3}})} + \frac{(K_{\text{P1}}[\text{H}^+]^2 + 2K_{\text{DPG2b}}[\text{H}^+] + 3K_{\text{P1}}K_{\text{DPG2b}}K_{\text{P3}})}{([\text{H}^+]^3 + K_{\text{P1}}[\text{H}^+]^2 + K_{\text{P1}}K_{\text{DPG2b}}[\text{H}^+] + K_{\text{P1}}K_{\text{DPG2b}}K_{\text{P3}})} \quad (7)$$

175 Albumin is modelled as described by Figge (13, 14). This particular model accounts
 176 for all charge associated with albumin, including aspartic and glutamic acids,
 177 tyrosine, lysine and arginine, cysteine, 16 histidine residues and the carboxy- and
 178 amino-termini, and the mean valence is given by:

$$z_{\text{Alb}} = -98 \frac{K_{\text{Asp/Glu}}}{(K_{\text{Asp/Glu}} + [\text{H}^+])} - 18 \frac{K_{\text{Tyr}}}{(K_{\text{Tyr}} + [\text{H}^+])} + 77 \left(1 - \frac{K_{\text{Lys/Arg}}}{(K_{\text{Lys/Arg}} + [\text{H}^+])} \right)$$

$$\begin{aligned}
& -\frac{K_{\text{Cys}}}{(K_{\text{Cys}} + [\text{H}^+])} + \sum_i^{16} \left(1 - \frac{K_{\text{AlbHis},i}}{(K_{\text{AlbHis},i} + [\text{H}^+])} \right) \\
& -\frac{K_{\text{AlbC}}}{(K_{\text{AlbC}} + [\text{H}^+])} + \left(1 - \frac{K_{\text{AlbZ}}}{(K_{\text{AlbZ}} + [\text{H}^+])} \right). \tag{8}
\end{aligned}$$

179

180 For haemoglobin, the mean valence depends upon whether the molecule is in the T or
 181 R state because the equilibrium constants differ between conformations. To
 182 determine the fraction of haemoglobin in each conformational state the oxygen
 183 saturation of haemoglobin must first be calculated. The function for saturation, S_{O_2} ,
 184 is that used by Radiometer (44), which itself is an updated version of the function
 185 proposed by Siggaard-Andersen *et al.* (50) and is a function of pH_p , PO_2 , PCO_2 ,
 186 fractions of carboxyhaemoglobin (f_{HbCO}), fetal haemoglobin (f_{HbF}), and
 187 methaemoglobin (f_{HbMet}), and concentration of DPG. For brevity this empirical
 188 function is expressed as:

$$S_{\text{O}_2} = f\{\text{pH}_p, P_{\text{O}_2}, P_{\text{CO}_2}, f_{\text{HbCO}}, f_{\text{HbF}}, f_{\text{HbMet}}, [\text{DPG}]_c\}, \tag{9}$$

189 but can be found in full in (44). This function is evaluated numerically. Once the
 190 oxygen saturation has been determined, the oxygenated, deoxygenated, relaxed, and
 191 tense fractions of haemoglobin (accounting for dyshaemoglobins) are determined
 192 from:

$$f_{\text{HbO}_2} = S_{\text{O}_2}(1 - f_{\text{HbMet}} - f_{\text{HbCO}} - f_{\text{HbF}}), \tag{10}$$

$$f_{\text{Hbdeoxy}} = (1 - S_{\text{O}_2})(1 - f_{\text{HbMet}} - f_{\text{HbCO}} - f_{\text{HbF}}), \tag{11}$$

$$f_{\text{HbR}} = f_{\text{HbO}_2} + f_{\text{HbCO}} + f_{\text{HbF}} + f_{\text{HbMet}}, \tag{12}$$

193 and

$$f_{\text{HbT}} = f_{\text{Hbdeoxy}}. \quad (13)$$

194 f_{HbMet} is normally considered to be an R-state (38), but this may be affected by
 195 phosphate concentration and pH, factors that we have not incorporated into the model.

196 The mean valence for the weakly ionised groups for haemoglobin in either its R or T
 197 state arises from six histidine residues in each alpha-chain, seven histidine residues in
 198 each beta-chain, and one amino-terminus per chain. The fractions of alpha-chain
 199 amino-termini that are in a protonated form or in a carbamino form for relaxed
 200 haemoglobin are given by:

$$f_{\text{HbR},\text{NH}_3^+,\alpha} = \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + K_{\text{HbZ},\text{R},\alpha}[\text{H}^+] + K_{\text{HbZ},\text{R},\alpha}K_{\text{HbC},\text{R},\alpha}[\text{CO}_2]}, \quad (14)$$

201 and

$$f_{\text{HbR},\text{NHCOO}^-,\alpha} = \frac{K_{\text{HbZ},\alpha}K_{\text{HbC},\alpha}[\text{CO}_2]}{[\text{H}^+]^2 + K_{\text{HbZ},\text{R},\alpha}[\text{H}^+] + K_{\text{HbZ},\text{R},\alpha}K_{\text{HbC},\text{R},\alpha}[\text{CO}_2]}. \quad (15)$$

202 The analogous expressions for the beta-chain amino termini for relaxed haemoglobin,
 203 and for both the alpha- and beta-chains for tense haemoglobin, are given by:

$$f_{\text{HbR},\text{NH}_3^+,\beta} = \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + K_{\text{HbZ},\text{R},\beta}[\text{H}^+] + K_{\text{HbZ},\text{R},\beta}K_{\text{HbC},\text{R},\beta}[\text{CO}_2]}, \quad (16)$$

$$f_{\text{HbR},\text{NHCOO}^-,\beta} = \frac{K_{\text{HbZ},\beta}K_{\text{HbC},\beta}[\text{CO}_2]}{[\text{H}^+]^2 + K_{\text{HbZ},\text{R},\beta}[\text{H}^+] + K_{\text{HbZ},\text{R},\beta}K_{\text{HbC},\text{R},\beta}[\text{CO}_2]}, \quad (17)$$

$$f_{\text{HbT},\text{NH}_3^+,\alpha} = \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + K_{\text{HbZ},\text{T},\alpha}[\text{H}^+] + K_{\text{HbZ},\text{T},\alpha}K_{\text{HbC},\text{T},\alpha}[\text{CO}_2]}, \quad (18)$$

$$f_{\text{HbT},\text{NHCOO}^-,\alpha} = \frac{K_{\text{HbZ},\alpha}K_{\text{HbC},\alpha}[\text{CO}_2]}{[\text{H}^+]^2 + K_{\text{HbZ},\text{T},\alpha}[\text{H}^+] + K_{\text{HbZ},\text{T},\alpha}K_{\text{HbC},\text{T},\alpha}[\text{CO}_2]}, \quad (19)$$

$$f_{\text{HbT},\text{NH}_3^+,\beta} = \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + K_{\text{HbZ},\text{T},\beta}[\text{H}^+] + K_{\text{HbZ},\text{T},\beta}K_{\text{HbC},\text{T},\beta}[\text{CO}_2]}, \quad (20)$$

204 and

$$f_{\text{HbT},\text{NHCOO}^-,\beta} = \frac{K_{\text{HbZ},\beta}K_{\text{HbC},\beta}[\text{CO}_2]}{[\text{H}^+]^2 + K_{\text{HbZ},\text{T},\beta}[\text{H}^+] + K_{\text{HbZ},\text{T},\beta}K_{\text{HbC},\text{T},\beta}[\text{CO}_2]}. \quad (21)$$

205

206 Using these fractions, the mean valence for relaxed haemoglobin is given by:

$$207 \quad z_{\text{HbR}} = \sum_i 2 \frac{[\text{H}^+]}{[\text{H}^+] + K_{\text{HbHis,R},i}} + 2 \left(f_{\text{HbR,NH}_3^+,\alpha} + f_{\text{HbR,NH}_3^+,\beta} \right) \\ - 2 \left(f_{\text{HbR,NHCOO}^-,\alpha} + f_{\text{HbR,NHCOO}^-,\beta} \right). \quad (22)$$

208 and for tense haemoglobin is given by:

$$209 \quad z_{\text{HbT}} = \sum_i 2 \frac{[\text{H}^+]}{[\text{H}^+] + K_{\text{HbHis,T},i}} + 2 \left(f_{\text{HbT,NH}_3^+,\alpha} + f_{\text{HbT,NH}_3^+,\beta} \right) \\ - 2 \left(f_{\text{HbT,NHCOO}^-,\alpha} + f_{\text{HbT,NHCOO}^-,\beta} \right). \quad (23)$$

210 The total charge concentration for the weakly ionised groups on haemoglobin is then

211 calculated from:

$$q_{\text{Hb}} = (z_{\text{HbR}} f_{\text{HbR}} + z_{\text{HbT}} f_{\text{HbT}}) \frac{[\text{Hb}]_b}{f_c}. \quad (24)$$

212

213 *Physical Constraints for Plasma and Intracellular $[\text{H}^+]$*

214 The previous section provides expressions for all the chemical moieties on the right-

215 hand side of *Eq. 1* and 2 in terms of known values apart from the values for plasma

216 and intracellular $[\text{H}^+]$. The approach we adopt for calculating plasma $[\text{H}^+]$ and

217 intracellular $[\text{H}^+]$ is an adaptation of Stewart's approach (54) and bears many

218 similarities to the method adopted by Wooten (62). It is based on the requirement for

219 electroneutrality within both the plasma and intracellular compartments.

$$220 \quad 0 = [\text{SID}]_p + [\text{PSID}]_p + [\text{H}^+]_p - [\text{HCO}_3^-]_p - 2[\text{CO}_3^{2-}]_p - [\text{OH}^-]_p$$

$$-z_{\text{Alb}}[\text{Alb}^{-z_{\text{Alb}}}]_p - z_{\text{Phos}}[\text{Phos}^{-z_{\text{Phos}}}]_p, \quad (25)$$

221 and

$$222 \quad 0 = [\text{ISID}]_c + [\text{PSID}]_c + [\text{H}^+]_c - [\text{HCO}_3^-]_c - 2[\text{CO}_3^{2-}]_c - [\text{OH}^-]_c - q_{\text{Hb}} \\ - z_{\text{Phos}}[\text{Phos}^{-z_{\text{Phos}}}]_c - z_{\text{DPG}}[\text{DPG}^{-z_{\text{DPG}}}]_c, \quad (26)$$

223 where ISID and PSID are the impermeant and permeant strong ion differences
 224 (meq.l^{-1}). These equations are very similar to Stewart's, but in our case there are two
 225 terms for strong ion difference. The impermeant strong ion differences are parameters
 226 of blood which do not change with PCO_2 and PO_2 . The manner in which these are set
 227 is described in "*Setting the Blood Parameters*" below. The permeant strong ion
 228 differences are variables of the model, and reflect the fact that there is an anionic
 229 exchange, known as the Cl^- or Hamburger shift (17) across the red cell membrane as
 230 CO_2 is loaded and unloaded from the blood. Apart from these two variables – PSID_p
 231 and PSID_c – and $[\text{H}^+]_p$, $[\text{H}^+]_c$, all the other variables on the right hand side of Eq. 25
 232 and 26 have already been defined (Eq. 3–8, 24).

233 In order to complete the specification of the system, we require two further
 234 constraints so that there are four equations from which to calculate the four variables:
 235 $[\text{H}^+]_p$, $[\text{H}^+]_c$, PSID_p , and PSID_c . The first constraint arises from the conservation of
 236 mass. This dictates that, while the compartmental variables PSID_p and PSID_c may
 237 vary over time, the permeant strong ion difference for whole blood, PSID_b , is a
 238 constant. This is another parameter of blood and is dependent on the total amount of
 239 chloride present. Thus we may write:

$$\text{PSID}_b = -[\text{Cl}^-]_b = (f_p[\text{PSID}]_p + f_c[\text{PSID}]_c). \quad (27)$$

240 The second constraint arises because both chloride and hydroxyl ions are freely
 241 permeant across the erythrocyte cell membrane, and therefore both species are
 242 distributed according to the Gibbs-Donnan equilibrium (55, 56, 59). As a
 243 consequence of the equilibrium between hydrogen ions and hydroxyl ions, hydrogen
 244 ions are also distributed according to the Gibbs-Donnan equilibrium, and we may
 245 write:

$$\frac{[\text{H}^+]_p}{[\text{H}^+]_c} = \frac{1}{0.85} \frac{[\text{Cl}^-]_c}{[\text{Cl}^-]_p} = \frac{1}{0.85} \frac{[\text{PSID}]_c}{[\text{PSID}]_p}, \quad (28)$$

246 where the factor of 0.85 results from the conversions between molarity, molality, and
 247 activity (NB: $[\text{H}^+]$ is defined as an activity, whereas other constituents are defined in
 248 terms of their molar concentrations), and allows for differences in ionic strength
 249 between the two compartments. For a fuller discussion of the matter see Siggaard-
 250 Anderson p81 (51).

251 This section has now established four equations (*Eq. 25–28*) from which the four
 252 variables $[\text{H}^+]_p$, $[\text{H}^+]_c$, PSID_p and PSID_c can in principle be solved.

253 *Setting the Blood Parameters*

254 The physical constants of the model are reported in Table 1. The equilibrium
 255 constants for the histidine residues in haemoglobin were originally reported at 29 °C
 256 (12) and have been correct to 37 °C using the van 't Hoff relation:

$$\ln \frac{K_2}{K_1} = -\frac{\Delta H^\circ}{R_0} \left(\frac{1}{T_2} - \frac{1}{T_1} \right), \quad (29)$$

257 where ΔH° is the standard enthalpy of ionization and is given for these groups in
 258 haemoglobin as 44.5 kJ.mol⁻¹ (62).

The parameters of blood that are required for the solution of the model are: haemoglobin concentration ($[\text{Hb}]_b$), cell fraction (f_c), plasma fraction (f_p), fractions of carboxyhaemoglobin (f_{HbCO}), fetal haemoglobin (f_{HbF}), and methaemoglobin (f_{HbMet}), ISID_p , ISID_c , PSID_b , and concentrations of plasma albumin, plasma phosphate and intra-erythrocytic 2,3-DPG. All of these parameters are either available as standard values for blood, or their values may be calculated directly from such values. Experimentally, most of the important values can be determined for a particular individual from a standard venous blood gas analysis that includes oximetry and that is coupled with simultaneous measurement of standard plasma electrolytes and, optionally, a measurement of plasma albumin. Default values for the parameters of blood that are used in the model are listed in Table 2, together with standard values for the components of blood on which these are based.

The fractional volume of the erythrocytic compartment, f_c , is set to be the same value as haematocrit (Hct). If the haematocrit is unknown, it is possible to use an empirical relationship for haematocrit in terms of the haemoglobin concentration in whole blood, as given by (29):

$$f_c = 0.0485(4[\text{Hb}]_b) + 0.0083. \quad (30)$$

The plasma fraction, f_p , is given by:

$$f_p = 1 - f_c. \quad (31)$$

Values for f_{HbCO} , f_{HbMet} , and f_{HbF} are relatively minor terms but are available directly from a blood gas measurement employing direct co-oximetry.

279 A set of standard values for the components of arterial blood is given in Table 2.

280 From these, we may calculate $ISID_p$ as:

$$[ISID]_p = [Na^+]_p + [K^+]_p + 2[Mg^{2+}]_p + 2[Ca^{2+}]_p - 2[SO_4^{2-}]_p - [Lac^-]_p. \quad (32)$$

281 Although lactate is to some degree permeant (24), it is treated here as impermeant
282 because its movement across the membrane is too slow to contribute to erythrocyte
283 anionic exchange during CO_2 loading/unloading in the tissues/lungs.

284 $PSID_p$, which is not a parameter of the model because it will vary with PCO_2 / PO_2 , is
285 given as:

$$PSID_p = -[Cl^-]_p, \quad (33)$$

286 Note that the sum of $ISID_p$ and $PSID_p$ gives a standard formulation for strong ion
287 difference of Stewart. Assuming a blood gas analysis is associated with these values
288 to give PCO_2 and pH_p , then every term in *Eq. 25* may be evaluated, which leads to a
289 degree of overspecification such that *Eq. 25* is only accurate to the level with which
290 the components of blood have been determined. To avoid this, it is best to use *Eq. 25*
291 to calculate one of the components in terms of all the others. If, as is commonly the
292 case, values for all the strong electrolytes are known but the plasma albumin is not,
293 then the concentration of albumin required for electro-neutrality can then be
294 calculated via *Eq. 25* and 8. This is possible because the model of Figge for albumin
295 (*Eq. 8*) includes all charged groups (both weakly and strongly ionised), and it is this
296 particular approach that was used in the present study.

297 The electro-neutrality constraint for erythrocytic fluid is also employed for initialising
298 the model in a manner similar to that for the extracellular fluid. However, while the

299 PCO₂ for the erythrocytic fluid is known from the blood gas data, the erythrocytic pH
 300 is not. However, this may be estimated from the following empirical relationship (52):

$$\text{pH}_c = 7.19 + 0.77(\text{pH}_p - 7.4). \quad (34)$$

301 The value for PSID_c (not a parameter of the model) may now be calculated from the
 302 Gibbs-Donnan equilibrium system (Eq. 28) as:

$$\text{PSID}_c = -[\text{Cl}^-]_c = -0.85[\text{Cl}^-]_p \frac{[\text{H}^+]_p}{[\text{H}^+]_c}. \quad (35)$$

303 The two values of PSID_p and PSID_c enable the calculation of the parameter PSID_b as
 304 per Eq. 27.

305 Given the value for PSID_c, and the values for PCO₂ and PO₂, and using the total
 306 concentrations for all the weakly ionised intracellular species (Table 2), Eq. 3–7 and
 307 9–24 can be used to evaluate all the terms in the electro-neutrality constraint for the
 308 erythrocytic fluid (Eq. 26) apart from ISID_c. Thus Eq. 26 may be used to calculate
 309 ISID_c, which completes the process of setting the parameters associated with blood.

310 *Solution method*

311 The section “*Physical Constraints for Plasma and Intracellular [H⁺]*” formulates four
 312 non-linear equations involving the four variables of [H⁺]_p, [H⁺]_c, PSID_p, and PSID_c
 313 which require solving to provide values for [H⁺]_p and [H⁺]_c. Once [H⁺]_p and [H⁺]_c
 314 have been found, it is then simple to calculate t_{CO₂} and t_{O₂} as defined by Eq. 1 and 2.

315 In principle, these four equations may be solved simultaneously using any of a
 316 number of standard equation solvers. However, the form of these equations is such
 317 that it is possible to eliminate sequentially PSID_c, PSID_p, and [H⁺]_c so as to have a
 318 single equation in [H⁺]_p. Although this would be algebraically unwieldy, this is

relatively simple to achieve computationally. It is the second approach that we used in this study.

The above procedure describes the forward solution of the model, that is, for a given PCO_2 and PO_2 , calculate t_{CO_2} and t_{O_2} . It is also possible to solve the inverse problem by embedding the forward routine in a further numerical routine. Here, the desired values for t_{CO_2} and t_{O_2} are specified, and initial guesses are supplied for PCO_2 and PO_2 . The model was implemented in MATLAB version 2014b. The numerical routine “fzero” was used for the forward problem. This routine was embedded within the numerical routine “fsolve” when solutions to the inverse problem were required.

Initial checks were performed by varying the PCO_2 and PO_2 to determine whether the changes observed were sensible and broadly in line with physiological expectations. The more detailed validation of the model against some of the more established observations from the literature is described in the Results.

Results

The outputs from the model over a range of PCO_2 values are illustrated in Fig. 1 for both oxygenated and deoxygenated blood. Shown are the dissociation curves, the lines on a Davenport diagram and a plot of the chemical moieties that make up the Haldane effect as a function of PCO_2 . In broad qualitative terms, the relationships all appear as expected. Around half of the Haldane effect arises from the formation of carbamino compounds and the other half from the increase in bicarbonate concentration.

For the purposes of model validation, values from the literature were selected for a number of different characteristics of blood, and these values are compared with the corresponding model outputs in Table 3. In general, the agreement between these values is either good, or very good. For arterial blood with a PCO_2 of 40 mmHg and a PO_2 of 100 mmHg, the model returns a value of 44.6 ml/100ml blood. The magnitude of the Haldane effect from the model is extremely similar to a summary of the literature values (58). The difference of 1% is well within the bounds of experimental error. The same is true for the Haldane coefficient, which is the number of moles of CO_2 exchanged per mole of O_2 exchanged at constant PCO_2 . Values for the absolute magnitudes for the slopes of lines on the Davenport diagram are of the order of 10% below those given by Siggaard-Andersen p47 (51). The model predicts that the line for deoxygenated blood is a little less steep than that for oxygenated blood and thus the lines converge for decreasing pH, and this is a detail that agrees with previously reported findings (30). The model value for the separation between the oxygenated and deoxygenated lines agrees very well with that reported by Lloyd and Michel (30). Finally, the relative change in pH within the erythrocyte as compared to the change in pH in plasma as CO_2 is loaded or removed agrees extremely well with the value of 0.77 from the literature.

Figure 2 illustrates the contribution of each mode of CO_2 transport for a number of different physiological states. These include rest (75% mixed venous SO_2 , $R=0.80$ and $R=0.85$), moderate exercise (50% mixed venous SO_2 , $R=0.85$ and $R=0.90$) and hard exercise (25% mixed venous SO_2 , $R=0.90$ and $R=0.95$, with 8 mM of lactic acid causing a reduction in $ISID_p$). The relative contributions to CO_2 transport are similar across all conditions. However, for the small differences that are present, there is a modest reduction in the percentage contribution of the Haldane effect with increasing

R. Thus, the percentage contribution of the Haldane effect during hard exercise is 28.8% as compared with a figure of 36.4% for rest. Corresponding values from (26), as measured from their figures, are 27% and 38%. The Haldane coefficient is also very similar across conditions, but there is a small reduction in hard exercise associated with the increase in lactate.

Figure 3 illustrates the effects of hyperoxia; metabolic acidosis and alkalosis; and anaemia and polycythemia on each of the modes of CO₂ transport. Marked arterial hyperoxia (here 600 mmHg) generates a significant quantity of O₂ physically dissolved in the blood. As metabolic consumption of dissolved O₂ does not affect haemoglobin saturation, this gives rise to a reduction in Haldane coefficient, as illustrated in Fig. 3. Metabolic acidosis and alkalosis affect the protonation of the amino termini in haemoglobin. This reduces (acidosis) or increases (alkalosis) the availability of amino groups for carbamino compound formation giving rise to the difference in Haldane coefficient between metabolic acidosis and alkalosis illustrated in Fig. 3. Anaemia and polycythemia vary the fraction of the blood that is plasma and the fraction that is intra-erythrocytic. Fig. 3 illustrates the CO₂ carriage for a constant venous O₂ saturation of 75%, and the most marked feature is the difference between anaemia and polycythemia in the arteriovenous difference for total CO₂. The relative contribution of bicarbonate formation through the Haldane effect is somewhat higher in anaemia than polycythemia. This arises because there is a larger plasma volume in anaemia within which bicarbonate can distribute.

The role of the amino termini of the globin chains in CO₂ transport through carbamino compound formation and H⁺ buffering is explored in Fig. 4 and Table 4. Carbamino compound formation comprises ~50% of the Haldane effect. However,

390 removal of the formation of carbamino compounds only reduces the magnitude of the
391 Haldane effect by 15% rather than 50% (Fig. 4B, Table 4). Two reasons lie behind
392 this. First, for each molecule of CO₂ bound to form a carbamino group, one amino
393 group is removed from the pool of H⁺ buffers, and second, the carbamino compound
394 itself strongly dissociates and therefore produces a further proton that requires
395 buffering. Thus in the absence of these additional H⁺ ions generated by carbamino
396 compound formation, bicarbonate formation increases as illustrated in Fig. 4B.

397 The effect of removing both carbamino compound formation and buffering by these
398 terminal amino groups still only reduces the Haldane effect by 23% (Fig. 4E,
399 Table 4). This reflects the prime importance to the Haldane effect of the shift in pK
400 with oxygenation of the histidine residues within the globin chains. Both the removal
401 of the carbamino compound forming ability and the removal of the buffering capacity
402 from the terminal amino groups increases the separation of the lines for oxygenated
403 and deoxygenated haemoglobin on the Davenport diagram (Fig. 4C, F). The
404 magnitude of this separation is commonly seen as a reflection of the magnitude of the
405 Haldane effect, but here the increase in separation is associated with a reduction in the
406 size of the Haldane effect.

407 Binding of chloride ions to haemoglobin has been associated with the transition of
408 haemoglobin from the R to the T state. This was explored in the model by varying the
409 charge distribution between total PSID and ISID_c with the saturation of haemoglobin
410 so as to mimic the degree of binding reported for haemoglobin in solution by Prange
411 *et al.* (42). This increases the magnitude of the Haldane effect, almost entirely through
412 an increased effect on bicarbonate (Fig. 5B). It also increases the separation between

oxygenated and deoxygenated lines on the Davenport diagram (Fig. 5C), but has almost no effect on the slopes of these lines (Table 4).

Finally we explored the effect of removing the chloride (Hamburger) shift from the model. This reduced the slope of the CO₂ dissociation curve in the physiological range (Fig. 5D), it reduced the magnitude of the Haldane effect through a reduction in bicarbonate formation (Fig. 5E, Table 4) and it reduced the slopes and removed the separation of the oxygenated and deoxygenated lines on the Davenport diagram (Fig. 5F, Table 4).

Discussion

We have shown that our current understanding of the physical chemistry of the major components of blood is both sufficiently good and sufficiently complete to predict the CO₂-carrying properties of whole blood with considerable accuracy. This was achieved by constructing a fully mechanistic model of the chemical equilibria both in the plasma and inside the red blood cell, while at the same time allowing exchange of permeant species across the erythrocyte membrane according to the governing physicochemical principles. The mechanistic nature of the model allowed the “in-silico” exploration of aspects of CO₂ carriage in a way that is not possible with simple, descriptive models of CO₂ carriage. In particular, we show that carbamino-haemoglobin compound formation adds very little to the CO₂-carrying capacity of haemoglobin because there is a concomitant reduction in bicarbonate formation resulting from a reduced proton-buffering capacity. We show that the putative binding of chloride ions to deoxygenated haemoglobin does not add significantly to CO₂ carriage, although it would significantly alter the distribution of bicarbonate across the red cell membrane, and consequently the separation of the lines for oxygenated

and deoxygenated blood on the Davenport diagram. Finally we show removal of the chloride (Hamburger) shift reduces both the slope of the CO₂ dissociation curve and the magnitude of the Haldane effect.

An early model of the rates of reaction of respiratory gases with blood was that of Hill *et al.* (22). This model also explored the significance of the Haldane effect, and concluded that it accounted for 46.4% of CO₂ exchange – a figure significantly greater than either ours of 36.4% or that of 38% (26) for resting conditions. One possible explanation is that, at that time, Hill *et al.* would have been unaware of the presence of carbonic anhydrase on the pulmonary capillary wall, and so modelled the plasma hydration of CO₂ in the absence of carbonic anhydrase. However, simulating this scenario with our model did not explain the difference. An alternative explanation is that there is an error in their model with respect to the Gibbs-Donnan ratio. They calculated this using plasma [H⁺] which will not be correct if disequilibrium within the CO₂-bicarbonate system is present. In support of this conclusion are the very different ratios between the plasma and red cell compartments for the variation in bicarbonate with PCO₂ under conditions when simultaneous O₂ exchange is absent as compared with when it is present.

A similar approach to ours for modelling whole blood, but limited to the fully oxygenated case for the purposes of understanding acid-base parameters, has been developed by Wooten (62). Apart from the restriction to fully oxygenated blood, a further difference from our model is that carbamino compound formation was neglected. Wooten also made a number of approximations including linearizing a number of equations, assuming a constant Gibbs-Donnan ratio and assuming a constant difference between plasma and intraerythrocytic pH which enabled him to

produce explicit linear equations for the variables he was seeking to calculate. Despite these differences, the buffer slope for whole blood obtained (29 mmol/l/pH) is not dissimilar from the value of 25.6 mmol/l/pH obtained in the present study.

Along with the present study, both that of Wooten *et al.* (62) and Rees (46) were based on Stewart's approach to the pH of biological fluids. Recently, there has been considerable debate concerning the usefulness of Stewart's approach vis-à-vis a traditional approach to the physiology of acid base (2, 25, 48, 57). A traditional approach to acid-base has been one of understanding changes in pH induced either by adding acid or alkali, or by varying PCO₂, with a particular consideration of the role of buffers in this process. Implicit in this approach are the notion of a starting pH, and the idea of titration of acid or alkali (or CO₂) that generates reactions involving protons resulting in a change in pH. The reactions of protons to generate changes in pH are clearly causal, because without reactions involving protons, the pH cannot change (assuming the volume remains constant). This has been, and remains, a useful way of understanding changes in pH. In Stewart's approach, there is no titration and there is no net reaction of protons. Here, the idea is that, given the PCO₂ of the solution, the strong ion difference and the total weak acid present, it is possible to calculate the pH of the solution. In this setting, the pH itself cannot be caused by net reactions of protons with buffers, because the system itself is in steady state. Thus Stewart's approach provides a powerful way to understand the states before and after titration, while traditional acid-base approaches provide a mechanistic way to understand the changes occurring during titration.

The removal of the chloride, or Hamburger, shift from our model had a number of discernible effects on CO₂ carriage. Bidani (8) also explored the effects of removing

485 the chloride shift from their model and found that the widening of the arteriovenous
486 difference for PCO_2 , from 6.0 to 8.3 mmHg, required to maintain constant CO_2
487 excretion was surprisingly small. In order to compare our findings with Bidani, we
488 conducted a similar simulation and found that the arteriovenous difference widened
489 from 6.0 to 7.9 mmHg. While these results are not quantitatively identical, our
490 qualitative conclusions are the same as those of Bidani.

491 While the present model for carbon dioxide transport by blood has been built entirely
492 from physicochemical principles, a limitation of the current work is that the model for
493 oxygen transport from which the percentage of haemoglobin in the R and T states has
494 been determined is simply phenomenological in nature. That the binding of oxygen to
495 haemoglobin is co-operative is evidenced by the sigmoid nature of the haemoglobin
496 dissociation curve. However, this binding is not completely co-operative because the
497 Hill coefficient (21) is lower than 4 which would be theoretical maximum for a
498 tetramer. Adair proposed a model where each intermediate state for the binding of
499 oxygen to tetrameric haemoglobin exists, but the binding of each successive oxygen
500 molecule became progressively easier (1). However, it has proved extremely difficult
501 to identify each of the individual rate constants, and furthermore the existence of the
502 Bohr effect means that these are not constants, but values that are affected by the
503 presence of other ligands, in particular protons, carbon dioxide and organic
504 phosphate. A more recent detailed discussion of these matters can be found in Dash
505 *et al.* (10).

506 Haemoglobin was one of the very first proteins to have its structure solved by x-ray
507 crystallography (39), but it took a further 10 years to complete the structure of both
508 the oxygenated and deoxygenated forms (41). Of particular note is that it exists in

509 two quaternary structures, a tense (T) deoxygenated form and a relaxed (R)
510 oxygenated form. This work beautifully illustrated the concept of allostery developed
511 by Monod, Wyman and Changeux (34), and resulted in haemoglobin being
512 considered as an “honorary enzyme”. Here, the binding of oxygen can be modelled
513 by equilibrium constants for the structural change between the tense and relaxed
514 forms whose values depend on how many molecules of oxygen are ligated to
515 haemoglobin. For myoglobin and isolated human chains, there is no Bohr effect. This
516 suggests that, rather than influencing the binding of oxygen, carbon dioxide and
517 protons influence the conformational change between T and R states directly. The
518 particular significance for the current work is that percentage of haemoglobin
519 molecules in the T and R states is only approximated through the saturation of the
520 blood with oxygen, and this is a significant limitation of our model. The
521 physicochemical modelling of all this is complex and incompletely resolved, but such
522 models are required if more complete predictions of the physiological properties of
523 whole blood are to be made from basic physicochemical principles.

524 In 1967, Benesch and Benesch described the importance of organic phosphates, and
525 in particular the importance of 2,3-DPG, in determining the P_{50} of haemoglobin (5, 6).
526 2,3-DPG preferentially binds to haemoglobin in its tense, deoxygenated form. A
527 binding site has been defined where the phosphate groups interact with the amino
528 termini of the β haemoglobin chains together with the His(2) residues of these chains
529 (4, 41). This suggests a binding stoichiometry of 1:1 for 2,3-DPG with haemoglobin,
530 but the use of higher concentrations of 2,3-DPG suggests a possible stoichiometry of
531 2:1 (15). The location of the second binding site is unclear. Of particular note to the
532 Haldane effect are that: i) 2,3-DPG is an effective proton buffer in its own right (23),
533 and preferential binding of 2,3-DPG to the tense, deoxygenated form of haemoglobin

will reduce the free concentration of 2,3-DPG; and ii) the binding sites of 2,3-DPG, at the amino termini of the β -chains, and at the His(2) residues of these chains, will interfere with the differential binding of both carbon dioxide and protons between the oxygenated and deoxygenated states. It has been suggested that 2,3-DPG binding underlies a component of the Haldane effect (47), but the uncertainties surrounding the various effects on proton binding are such that we have been unable to incorporate it into the present model. It is perhaps surprising that the present model reproduces the Haldane effect so effectively without taking into account the differential binding of 2,3-DPG between deoxygenated and oxygenated haemoglobin.

A further allosteric regulator of haemoglobin is chloride which appears to associate preferentially with the positive charges in the central cavity of deoxygenated haemoglobin (37, 40). A functional study demonstrated a small reduction in chloride concentration with deoxygenation of a haemoglobin solution, but this could not be detected for solutions containing red blood cells (42). One possible explanation is that, as well as being bound to deoxygenated haemoglobin, chloride is also displaced by the increased binding of 2,3-DPG, and these two effects cancel each other when haemoglobin is inside red blood cells. The importance of varying chloride binding to the present study is that chloride constitutes the permeant strong ion difference, and the binding of chloride to haemoglobin removes negative charge from the permeant strong ion difference and transfers it to the erythrocytic impermeant strong ion difference. This will alter the Gibbs-Donnan ratio across the erythrocyte membrane. In the model, we explored this effect using the chloride binding observed with the haemoglobin solution (42) and found that, while it widened the pH difference between oxygenated and deoxygenated lines on the Davenport diagram, it had little functional effect on CO₂ transport. While the physiological significance remains

unclear, this result demonstrates that the separation between the oxygenated and deoxygenated lines on the Davenport diagram need not necessarily all be related to the liberation of protons associated with the Haldane effect.

A further limitation of our model is that we did not incorporate governing equations to ensure osmotic equilibrium between plasma and erythrocytic fluid. While shifts in water between erythrocytic fluid and plasma are small, they nevertheless occur (51). An approach to solving this particular problem has been given by Wolf (61) and Wolf and DeLand (60).

In summary, we have presented a physicochemical model of carbon dioxide carriage by blood that has been coupled with an accepted phenomenological model for oxygen carriage. The model has focussed on the binding of protons and carbon dioxide to various constituents of blood and on the anionic exchange across the red blood cell membrane. Despite certain limitations, the model has produced an accurate prediction of CO₂ carriage by blood in general, and the Haldane effect in particular. Hopefully the century of progress that has followed the discovery of the Haldane effect will soon enable a fully-integrated model of whole blood where oxygen carriage can similarly be modelled using just the underlying physicochemical principles.

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757 **Figure legends**

758

759 Figure 1. Results from standard model

760 A, total carbon dioxide, t_{CO_2} , plotted against PCO_2 for oxygenated and deoxygenated
761 whole blood. B, contributions of bicarbonate and carbamino compound formation to
762 the Haldane effect between oxygenated and deoxygenated blood plotted against PCO_2 ;
763 C, $[HCO_3^-]_p$, pH_p relationship for oxygenated and deoxygenated blood (Davenport
764 diagram). Values of the slopes of the lines and the pH separation are given in
765 Table 3.

766

767 Figure 2. Arterio-venous differences for CO_2 transport under different steady-state
768 physiological conditions (I).

769 A, change in total CO_2 carriage. B, percentage contribution of different forms of CO_2
770 carriage. C, Haldane coefficient, which gives the number of moles of CO_2 exchanged
771 by the Haldane effect per mole of O_2 exchanged. Shown are example values for rest
772 ($S_{\bar{v}O_2} = 75\%$), moderate exercise ($S_{\bar{v}O_2} = 50\%$) and heavy exercise ($S_{\bar{v}O_2} = 25\%$, with a
773 plasma lactate concentration of 8 mmol.l^{-1} and reduced $P_{aCO_2} = 30 \text{ mmHg}$).
774 Examples are given for a range of possible values of R . Note the percentage

775 contributions to CO₂ transport of the various mechanisms and the values for the
 776 Haldane coefficient are relatively stable across these different conditions.
 777

778 Figure 3. Arterio-venous differences for CO₂ transport under different steady-state
 779 physiological conditions (II).
 780 *A, B, C*, as for Fig. 2. Shown are standard conditions at rest; the effect hyperoxia
 781 (arterial PCO₂ = 600 mmHg); the effects of metabolic acidosis and alkalosis p46 (43)
 782 and the effects of anaemia (Hb reduced 50%) and polycythemia (Hb increased 50%).
 783 Note the variations in percentage contributions to CO₂ transport of the various
 784 mechanisms and the associated variations in Haldane coefficient.
 785

786 Figure 4. Role of terminal amino groups of the globin chains in CO₂ carriage by the
 787 blood

788 *A, B*, and *C*, effects of eliminating carbamino compound formation by terminal amino
 789 groups; *D, E*, and *F*, effects of eliminating both carbamino compound formation and
 790 H⁺ buffering by the terminal amino groups. *A* and *D*, total carbon dioxide, *t*_{CO₂},
 791 plotted against PCO₂ for oxygenated and deoxygenated whole blood. The short dotted
 792 lines indicate results from the original standard model as shown in Fig. 1. *B* and *E*,
 793 size of Haldane effect between oxygenated and deoxygenated blood plotted against
 794 PCO₂. In the absence of carbamino compound formation, differences in bicarbonate
 795 comprise the whole of the Haldane effect. Lower (white) dotted lines indicate the
 796 contribution of bicarbonate to the Haldane effect, and upper (black) dotted lines
 797 indicate the magnitude of the total Haldane effect (bicarbonate plus carbamino

compound formation) for the standard model shown in Fig. 1. Note that, although the Haldane effect is less, the change in bicarbonate is substantially greater than in the presence of carbamino compound formation. C and F , $[\text{HCO}_3^-]_p$, pH_p relationship for oxygenated and deoxygenated blood. The separation of the two lines is greater in the absence of carbamino compound formation and the bar displays the separation observed for the standard model. Differences in the slope from the standard model are modest and are given in Table 4.

Figure 5. Role of chloride binding to haemoglobin and of the chloride (Hamburger) shift in CO_2 carriage by the blood

A , B , and C , effects of introducing chloride binding to deoxygenated haemoglobin; D , E , and F , effects of eliminating the chloride (Hamburger) shift. A and D , total carbon dioxide, t_{CO_2} , plotted against PCO_2 for oxygenated and deoxygenated whole blood. The short dotted lines indicate results from the original standard model as shown in Fig. 1; the line for oxygenated blood in A is obscured by the model output. B and E , size of Haldane effect between oxygenated and deoxygenated blood plotted against PCO_2 . Lower (white) dotted lines indicate the contribution of bicarbonate to the Haldane effect, and upper (black) dotted lines indicate the magnitude of the total Haldane effect (bicarbonate plus carbamino compound formation) for the standard model shown in Fig. 1. C and F , $[\text{HCO}_3^-]_p$, pH_p relationship for oxygenated and deoxygenated blood. The bars indicate the separation observed for the standard model in Fig. 1. In the case of chloride binding to haemoglobin (A , B , and C), the Haldane effect is greater through an effect on bicarbonate generation. The separation of the oxygenated and the deoxygenated lines in the Davenport diagram is enhanced

compared with the standard model. Differences in the slopes from the standard model are modest and are given in Table 4. In the case of removal of the chloride shift (D , E , and F), the Haldane effect is reduced through a reduction in bicarbonate generation. The separation of the oxygenated and deoxygenated line in the Davenport diagram is lost. The slope of these lines is reduced (Table 4).

Table 1. Physicochemical constants of model at 37 °C

Constant	Value	Units	Source
pK_{H_2O}	13.6		
$\alpha_{CO_2,p}$	2.2952×10^{-4}	mmol. Pa ⁻¹	(51)
$\alpha_{CO_2,c}$	1.9509×10^{-4}	mmol. Pa ⁻¹	(51)
$pK_{CO_2,1}$	6.104		(51)
$K_{CO_2,2}$	6.00×10^{-11}	l. mmol ⁻¹	(14)
K_{P1}	1.22×10^{-2}	l. mmol ⁻¹	(3)
K_{P2}	2.19×10^{-7}	l. mmol ⁻¹	(23)

K_{P3}	1.66×10^{-12}	l. mmol^{-1}	(3)
pK_{DPG2a}	6.39		(23)
pK_{DPG2b}	7.28		(23)
$K_{\text{Asp/Glu}}$	1.00×10^{-4}	l. mmol^{-1}	(3)
K_{Tyr}	2.51×10^{-10}	l. mmol^{-1}	(3)
$K_{\text{Lys/Arg}}$	3.98×10^{-10}	l. mmol^{-1}	(3)
K_{Cys}	3.16×10^{-9}	l. mmol^{-1}	(3)
$K_{\text{Alb,1}}$	7.58×10^{-8}	l. mmol^{-1}	(3)
$K_{\text{Alb,2}}$	6.03×10^{-8}	l. mmol^{-1}	(3)
$K_{\text{Alb,3}}$	7.94×10^{-8}	l. mmol^{-1}	(3)
$K_{\text{Alb,4}}$	3.23×10^{-8}	l. mmol^{-1}	(3)
$K_{\text{Alb,5}}$	9.77×10^{-8}	l. mmol^{-1}	(3)
$K_{\text{Alb,6}}$	4.90×10^{-8}	l. mmol^{-1}	(3)
$K_{\text{Alb,7}}$	1.78×10^{-7}	l. mmol^{-1}	(3)
$K_{\text{Alb,8}}$	4.37×10^{-7}	l. mmol^{-1}	(3)
$K_{\text{Alb,9}}$	1.41×10^{-5}	l. mmol^{-1}	(3)
$K_{\text{Alb,10}}$	1.74×10^{-6}	l. mmol^{-1}	(3)
$K_{\text{Alb,11}}$	6.76×10^{-7}	l. mmol^{-1}	(3)
$K_{\text{Alb,12}}$	1.86×10^{-7}	l. mmol^{-1}	(3)
$K_{\text{Alb,13}}$	1.51×10^{-6}	l. mmol^{-1}	(3)
$K_{\text{Alb,14}}$	5.01×10^{-8}	l. mmol^{-1}	(3)
$K_{\text{Alb,15}}$	6.31×10^{-6}	l. mmol^{-1}	(3)
$K_{\text{Alb,16}}$	5.01×10^{-8}	l. mmol^{-1}	(3)
K_{AlbC}	7.94×10^{-4}	l. mmol^{-1}	(3)
K_{AlbZ}	1.00×10^{-8}	l. mmol^{-1}	(3)
$K_{\text{HbC,R},\alpha}$	5.01×10^{-6}	l. mmol^{-1}	(36)
$K_{\text{HbC,R},\beta}$	5.01×10^{-6}	l. mmol^{-1}	(36)
$K_{\text{HbZ,R},\alpha}$	1.17×10^{-7}	l. mmol^{-1}	(36)
$K_{\text{HbZ,R},\beta}$	1.17×10^{-7}	l. mmol^{-1}	(36)
$K_{\text{HbC,T},\alpha}$	1.74×10^{-5}	l. mmol^{-1}	(36)
$K_{\text{HbC,T},\beta}$	2.88×10^{-5}	l. mmol^{-1}	(36)
$K_{\text{HbZ,T},\alpha}$	3.47×10^{-8}	l. mmol^{-1}	(36)
$K_{\text{HbZ,T},\beta}$	2.34×10^{-7}	l. mmol^{-1}	(36)
$K_{\text{HbHis,R},1}$	1.31×10^{-7}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},2}$	1.20×10^{-6}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},3}$	1.99×10^{-7}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},4}$	8.48×10^{-8}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},5}$	8.88×10^{-7}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},6}$	4.66×10^{-8}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},7}$	6.44×10^{-7}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},8}$	2.56×10^{-8}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},9}$	2.81×10^{-8}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},10}$	1.17×10^{-6}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},11}$	6.44×10^{-7}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},12}$	4.25×10^{-6}	l. mmol^{-1}	(12)
$K_{\text{HbHis,R},13}$	6.01×10^{-7}	l. mmol^{-1}	(12)

$K_{\text{HbHis,T},1}$	1.51×10^{-7}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},2}$	8.88×10^{-6}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},3}$	1.14×10^{-7}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},4}$	5.35×10^{-8}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},5}$	2.50×10^{-7}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},6}$	5.11×10^{-8}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},7}$	1.07×10^{-6}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},8}$	5.48×10^{-8}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},9}$	1.54×10^{-8}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},10}$	7.06×10^{-7}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},11}$	5.87×10^{-7}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},12}$	3.15×10^{-5}	l. mmol^{-1}	(12)
$K_{\text{HbHis,T},13}$	1.86×10^{-8}	l. mmol^{-1}	(12)
ΔH°	44.5	kJ. mol^{-1}	(62)
$\alpha_{\text{O}_2,b}$	1.020×10^{-5}	mmol. Pa^{-1}	(51)

837

838 Ref (12) provided values at 29 °C and these have been converted to 37 °C.

839 Table 2. Concentrations of chemical components of blood used in standard model.

Variable	Value	Units	Source
$[\text{Ca}^{2+}]_p$	2.1	mmol. l ⁻¹	(14)
$[\text{Mg}^{2+}]_p$	1.1	mmol. l ⁻¹	(14)
$[\text{K}^+]_p$	4.0	mmol. l ⁻¹	(3)
$[\text{Na}^+]_p$	140	mmol. l ⁻¹	(3)
$[\text{Cl}^-]_p$	105	mmol. l ⁻¹	(3)
$[\text{SO}_4^{2-}]_p$	0.8	mmol. l ⁻¹	(14)
$[\text{Lac}^-]_p$	0.7	mmol. l ⁻¹	(3)
$[\text{Phos}]_p$	1.16	mmol. l ⁻¹	(3)
$[\text{OrgPhos}]_p$	5.7	mmol. l ⁻¹	(3)
$[\text{DPG}]_c$	5.0	mmol. l ⁻¹	(3)
$[\text{Hb}]_b$	2.325	mmol. l ⁻¹	p356 (16)
f_{HbCO}	0.01		(44)
f_{HbMet}	0.0075		(44)
f_{HbF}	0.0		(44)

840

841 Table 3. Model outputs for CO₂ transport properties of blood.

Variable	Model output	Literature value	Deviation from literature
Total CO ₂ content of arterial blood $(t_{\text{CO}_2})_{P_{\text{CO}_2}=40\text{mmHg}, P_{\text{O}_2}=100\text{mmHg}}$ / ml.dl ⁻¹	44.6	46.4 (58)	-3.8%
Haldane effect $(\Delta t_{\text{CO}_2})_{P_{\text{CO}_2}=40\text{mmHg}}$ / ml.dl ⁻¹	6.34	6.28 eq 4 (58)	+1.0%
Haldane coefficient $R=0.8, S_{\bar{v}\text{O}_2}=75\%$	-0.29	-0.25 to -0.30 (28)	Within range
Davenport slope for oxygenated blood $\left(\frac{\partial[\text{HCO}_3^-]_p}{\partial \text{pH}_p}\right)_{P_{\text{O}_2}=200\text{mmHg}}$ / mmol.l ⁻¹	-25.6	-28.0 p47 eq10 (51)	-8.6%
Davenport slope for deoxygenated blood $\left(\frac{\partial[\text{HCO}_3^-]_p}{\partial \text{pH}_p}\right)_{P_{\text{O}_2}=2\text{mmHg}}$ / mmol.l ⁻¹	-24.7	-27.8 p47 eq10 (51)	-11%
Davenport diagram line separation $(\Delta \text{pH}_p)_{[\text{HCO}_3^-]_p=25\text{mM}}$	0.084	0.084 (30)	+0.7%
Gradient of pH-relationship line $\left(\frac{\partial \text{pH}_c}{\partial \text{pH}_p}\right)_{P_{\text{O}_2}=200\text{mmHg}}$	0.75	0.77 p77 (51)	-1.8%
Gradient of pH-relationship line $\left(\frac{\partial \text{pH}_c}{\partial \text{pH}_p}\right)_{P_{\text{O}_2}=2\text{mmHg}}$	0.77	0.77 p77 (51)	+0.4%

842

843 Table 4. Role of amino terminus of haemoglobin; putative Cl⁻ binding by
844 haemoglobin; and the chloride (Hamburger) shift on CO₂ transport by blood

Variable	Standard model output	No carbamino compound formation	No carbamino compound formation or H ⁺ buffering by amino termini	Standard model with Cl ⁻ binding to deoxygenated haemoglobin	Standard model with chloride (Hamburger) shift disabled
Total CO ₂ content of arterial blood $(t_{\text{CO}_2})_{P_{\text{CO}_2}=40\text{mmHg}, P_{\text{O}_2}=100\text{r}}$ / ml.dl ⁻¹	44.6	43.5	43.5	44.6	44.4
Haldane effect $(\Delta t_{\text{CO}_2})_{P_{\text{CO}_2}=40\text{mmHg}}$ / ml.dl ⁻¹	6.34	5.38	4.90	6.96	5.32
Haldane coefficient $R=0.8, S_{\bar{v}\text{O}_2}=75\%$	-0.29	-0.25	-0.23	-0.32	-0.24
Davenport slope for oxygenated blood $\left(\frac{\partial[\text{HCO}_3^-]_p}{\partial \text{pH}_p}\right)_{P_{\text{O}_2}=200\text{mmHg}}$ / mmol.l ⁻¹	-25.6	-26.3	-23.4	-25.7	-8.1
Davenport slope for deoxygenated blood $\left(\frac{\partial[\text{HCO}_3^-]_p}{\partial \text{pH}_p}\right)_{P_{\text{O}_2}=2\text{mmHg}}$ / mmol.l ⁻¹	-24.7	-25.9	-23.6	-24.9	-8.0
Davenport diagram line separation $(\Delta \text{pH}_p)_{[\text{HCO}_3^-]_p=25\text{mM}}$	0.084	0.132	0.129	0.137	0.007
Gradient of pH- relationship line $\left(\frac{\partial \text{pH}_c}{\partial \text{pH}_p}\right)_{P_{\text{O}_2}=200\text{mmHg}}$	0.76	0.75	0.79	0.76	0.41
Gradient of pH- relationship line $\left(\frac{\partial \text{pH}_c}{\partial \text{pH}_p}\right)_{P_{\text{O}_2}=2\text{mmHg}}$	0.77	0.76	0.79	0.76	0.48

845