Computer Methods and Programs in Biomedicine, 38 (1992) 195–226

COMMET 01305

Section II. Systems and programs

A Macintosh software package for simulation of human red blood cell metabolism

I-Der Lee and Bernhard O. Palsson

Cellular Biotechnology Laboratory, Department of Chemical Engineering, University of Michigan, Ann Arbor, MI, USA

We have developed a computer software package for Macintosh to simulate the metabolism and hemoglobin binding affinity of human red blood cell. The model is capable of simulating hemoglobin binding of ligands, metabolite concentrations, and metabolic fluxes at physiological steady state and in response to extracellular parameter variations, such as pH, osmolarity, glucose, and adenine concentrations. The kinetic parameters of enzymes, extracellular conditions, and initial intracellular metabolite concentrations can be specified by the user in order to model a particular situation. The software is use friendly, utilizing menu, window, and mouse to interact with the user. It also provides a pathway map of the red cell, which allows a direct access to enzyme kinetics by clicking the enzymes in the map.

Metabolic model; Human erythrocyte; Erythrocyte metabolism

1. Introduction

Red blood cells have been subjected to intensive studies due to their vital functions in human body. The basic physiological function of the red cell is to deliver oxygen and carbon dioxide between the lungs and tissues. The metabolism of the red cell is thus designed to regulate the hemoglobin binding affinity with the two gases and maintain the viability of the cell. The most important metabolites involved in the red cell functions are 23DPG and ATP, concentrations of which can be sufficiently supplied and regulated by glycolysis, pentose pathway, and nucleotide synthesis processes that constitute the red cell metabolism.

A large body of experimental data have been accumulated for the last three decades, providing detailed information about individual enzymes in the red cell. Efforts in collecting these information and organizing them into quantitative models for red cell metabolic pathways began in the early 1970s [1]. Several metabolic models for the red cell have since been developed, which mainly consider glycolysis and pentose pathway [1–9]. However, there was a lack of a comprehensive metabolic model which can be used to analyze the red cell metabolism as a whole living cell.

We thus set out to construct a full model for the red cell, which account for glycolysis, pentose pathway, nucleotide synthesis, cation transports, magnesium complexation, pH dependence of enzymes, hemoglobin binding, electroneutrality, and osmotic balance [10–12]. This model considers all major metabolic reactions, interaction between intracellular metabolism with extracellular perturbation, regulation of hemoglobin binding by its

Correspondence: Bernhard O. Palsson, Cellular Biotechnology Laboratory, Department of Chemical Engineering, University of Michigan, Ann Arbor, MI 48109-2136, USA.

ligands, and pH dependence of enzyme kinetics. We have verified the model calculation against literature experimental data; the model can predict the physiological steady state of red cell metabolism [11] and dynamic response of the red cell to an extracellular pH perturbation [12].

A software package that simulates the red cell metabolic model has been writen for Apple Macintosh, utilizing the friendly operating system of the computer to interact with users. The package allows the user to change kinetic parameters for enzymes, to calculate steady state metabolite concentrations and fluxes, dynamic response of the cell to extracellular perturbations, variation of each enzyme activity as functions of extra- and intracellular conditions, and hemoglobin binding curves of ligands.

This paper will describe the basic features of the mathematical model, the structure of the program, and the capability of the simulator, and finally provide several sample calculations.

2. Computational methods and theory

The red cell metabolic model accounts for 33 metabolites, 41 enzymatic reactions, 2 physicochemical constraints, and several chemical equilibrium reactions, which are individually calculated in separated subroutines. The model contains 33 ordinary differential equations with 33 metabolites concentrations as dependent variables. The kinetic rate laws and equations used to describe metabolic reactions, cofactor balances, membrane transport, osmotic balance, electroneutrality, Mg complex balance, and hemoglobin binding in the red cell are described as follows.

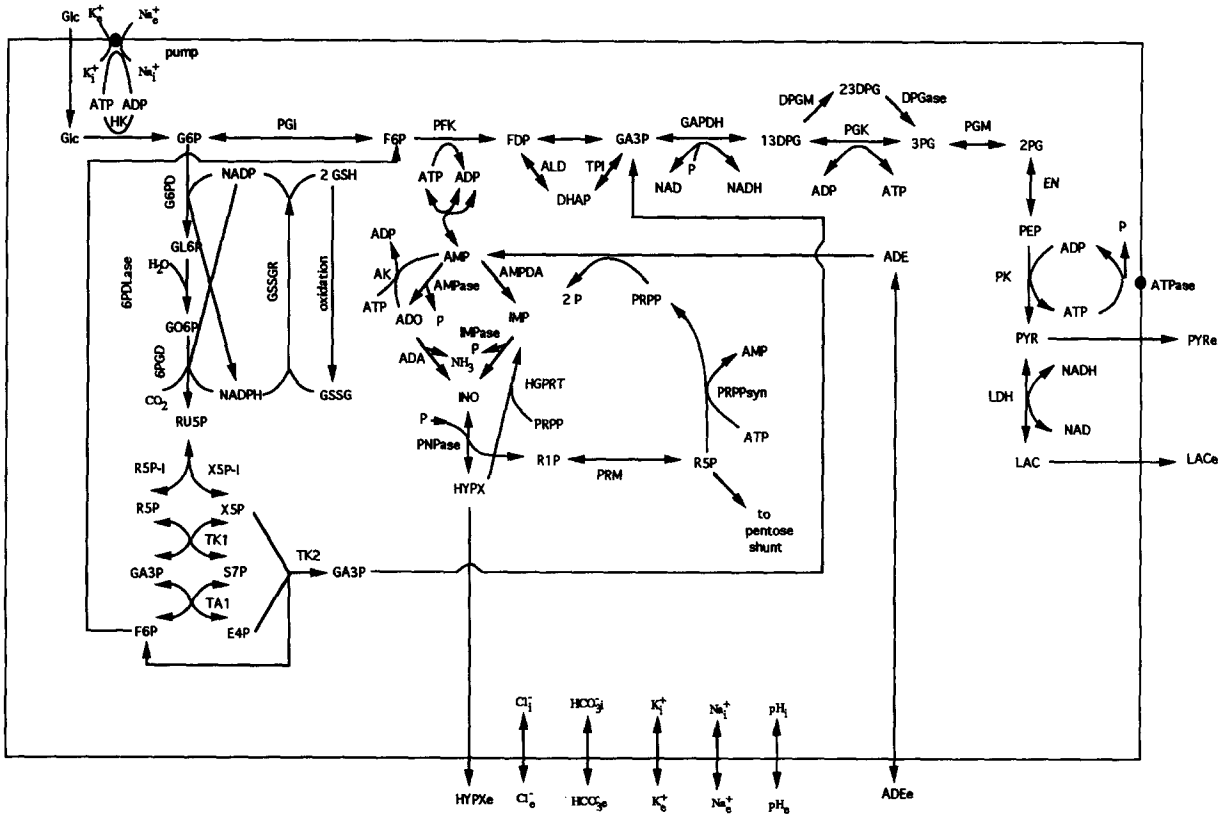


Fig. 1. The mathematical model of human red cell metabolism.

2.1. Metabolic reactions

The equations that describe the transient changes in metabolite concentrations are simply dynamic mass balances. The change in *amount* of a substance per cell per unit time is given by the sum of all the synthetic rates minus the sum of all degradation rates (see Fig. 1):

$$\frac{dVC_i}{dt} = V\sum_{j=1}^{n} \nu_{ij} v_{f_{ij}} - V\sum_{k=1}^{n} \nu_{ik} v_{d_{ik}} \qquad (1)$$

where C_i is the concentration of variable i; $v_{f_{ij}}$ is the jth synthetic rate of concentration variable i, and $v_{d_{ik}}$ is the kth degradation rate of concentration variable i (the v's represent volumetric reaction rates). The volume of the cell is represented by V and time by t. The stochiometric coeffi-

TABLE 1

Red cell intermediates and their abbreviations

Abbreviation	Metabolic intermediates
G6P	Glucose 6-phosphate
F6P	Fructose 6-phosphate
FDP	Fructose 1,6-diphosphate
DHAP	Dihydroxyacetone phosphate
GA3P	Glyceraldehyde 3-phosphate
3PG	3-phosphoglycerate
2PG	2-phosphoglycerate
PEP	Phosphoenolpyruvate
PYR	Pyruvate
LAC	Lactate
1,3DPG	1,3-Diphosphoglycerate
2,3DPG	2,3-Diphosphoglycerate
NAD	Nicotinamide adenine dinucleotide
ATP	Adenosine tri-phosphate
ADP	Adenosine di-phosphate
AMP	Adenosine mono-phosphate
ADO	Adenosine
ADE	Adenine
GL6P	6-Phosphoglucono-δ-lactone
GO6P	6-Phosphogluconate
NADP	Nicotinamide adenine phosphate
GSH	Glutathione
RU5P	Ribulose 5-phosphate
R5P	Ribose 5-phosphate
X5P	Xylulose 5-phosphate
S7P	Sedoheptulose 5-phosphate
E4P	Erythrose 4-phosphate
PRPP	5-phosphoribosyl-1-pyrophosphate
IMP	Inosine mono-phosphate
INO	Inosine
HX	Hypoxanthine
R1P	Ribose 1-phosphate
K	Potassium
Na	Sodium

TABLE 2

Red cell metabolic enzymes and their abbreviations

Abbreviation	Enzymes
HK	Hexokinase
PGI	Phosphoglucoisomerase
PFK	Phosphofructokinase
ALD	Aldolase
TPI	Triose phosphate isomerase
GAPDH	Glyceraldehyde phosphate dehydrogenase
PGK	Phosphoglycerate kinase
DPGM	Diphosphoglycerate mutase
DPGase	Diphosphoglycerate phosphatase
PGM	Phosphoglyceromutase
EN	Enolase
PK	Pyruvate kinase
LDH	Lactate dehydrogenase
PYRex	Pyruvate export
LACex	Lactate export
AMPase	Adenosine monophosphate phosphohydrolase
ADA	Adenosine deaminase
AK	Adenosine kinase
ApK	Adenylate kinase
AMPDA	Adenosine monophosphate deaminase
ATPase	Adenosine triphosphate phosphohydrolase
AdPRT	Adenine phosphoribosyl transferase
G6PDH	Glucose-6-phosphate dehydrogenase
6PGL	6-Phosphogluconolactonase
6PGLDH	6-Phosphogluconate dehydrogenase
GSSGR	Glutathione reductase
GSH	Glutathione oxidase
R5P-I	Ribose-5-phosphate isomerase
Xu5P-I	Xylulose-5-phosphate epimerase
TKI	Transketolase
TKII	Transketolase
TA	Transaldolase
IMPase	Inosine monophosphatase
PNPase	Purine nucleoside phosphorylase
PRM	Phosphoribomutase
PRPPsyn	Phosphoribosyl pyrophosphate synthetase
HGPRT	Hypoxanthine-guanine phosphoryl transferase
HXex	Hypoxanthine export
LeakK	Leak of potassium out of the red cell
LeakNa	Leak of sodium out of the red cell
Pump	Sodium/potassium pump

TABLE 3

The rate expressions used to describe the irreversible reactions in red cell glycolysis

The unit for a concentration is mM, and the unit for a reaction rate is mM/h. The references shown are for the pH dependence of kinetic constants. For the kinetic expressions, see [11].

Enzyme	Rate expression	Parameter values
Hexokinase (Schauer, 1981)	$v = \frac{1}{N}\left(v_1 \frac{Glc}{K_{MgATP,Glc}} \frac{MgATP}{K_{MgATP}} + V_2 \frac{Glc}{K_{MgATP,Glc}} \frac{MgATP}{K_{MgATP}} \frac{Mg}{K_{MgATP,Mg}}\right)$	$K_{MgATP,Glc} = 0.051$
	$N = 1 + \frac{Glc}{K_{Glc}} + \left(1 + \frac{Glc}{K_{MgATP,Glc}}\right)\frac{MgATP}{K_{MgATP}} + \left(1 + \frac{Glc}{K_{Glc}}\right)\frac{Mg}{K_{Mg}}$	$K_{MgATP} = 1.13$
	$+ \left(1 + \frac{Glc}{K_{MgATP,Glc}}\right)\frac{MgATP}{K_{MgATP}} + \left(1 + \frac{Glc}{K_{GLC}}\right)\frac{Mg}{K_{Mg}}$	$K_{MgATP,Mg} = 1.14$
	$+ \left[\left(1 + \frac{Glc}{K_{Glc}}\right)\frac{G6P}{K_{Glc,G6P}} + 1.55\right]\left(1 + \frac{Mg}{K_{Mg}}\right)$	$K_{Glc} = 0.04$
	$+ \left(1 + \frac{Glc}{K_{Glc}}\right)\left(\frac{2,3DPG}{K_{2,3DPG}} + \frac{Mg}{K_{Mg}}\frac{2,3DPG}{K_{Mg,2,3DPG}}\right)$	$K_{Glc,G6P} = 0.069$
	$V_1 = \frac{7.5095}{1 + \frac{H}{K_1} - \frac{K_2}{H}}$	$K_{Mg} = 1.03$
	$V_2 = 2.1187 V_1$	$K_{2,3DPG} = 2.7$ $K_{M,g,2,3DPG} = 3.44$ $K_1 = 2.2718 \cdot 10^{-4}$ $K_2 = 4.60 \cdot 10^{-6}$
Phosphofuctokinase (Schauer, 1981)	$v = \frac{V_m}{N}\frac{F6P}{K_{F6P} + F6P}\frac{MgATP}{K_{MgATP} + MgATP}$	$V_m = 250$
	$N = 1 + L\frac{(1 + ATP/K_{ATP})^4 (1 + Mg/K_{Mg})^4}{(1 + AMP/K_{AMP})^4 (1 + F6P/K_{F6P})^4}$	$K_{F6P} = 0.1$
	$K_{ATP} = e^{K_1 + K_2 PH}$	$K_{MgATP} = 0.068$ $K_{AMP} = 0.033$ $K_{Mg} = 0.44$ $L = 1.072 \cdot 10^{-3}$ $K_1 = 6.96644$ $K_2 = 0.32796$
Pyryvate kinase (Kilinc, 1984)	$v = \frac{V_m}{N}\frac{MgADP}{K_{MGADP} + MgADP}\frac{PEP}{K_{PEP} + PEP}$	$K_{ADP} = 0.474$
	$N = 1 + L\frac{(1 + ATP/K_{ATP})^4}{(1 + PEP/K_{PEP})^4 (1 + FDP/K_{FDP})^4}$	$K_{PEP} = 0.225$
	$V_m = 250 - 75.75\ (pH - 7.2)$ when $pH \geqslant 7.2$ $V_m = 250 + 94.67\ (pH - 7.2)$ when $pH \geqslant 7.2$	$K_{ATP} = 3.39$ $K_{FDP} = 0.005$ $L = 19$
Aldolase	$\frac{(N_1 A - N_2 PQ)ET}{D_1 + D_2 A + D_3 P + D_4 Q + D_5 AP + D_6 PQ}$	$ET = 0.37 \cdot 10^{-3}$
	where A = FDP, P = GAP, Q = DHAP	$K_{eq} = 0.081$

TABLE 3 (continued)

Aldolase		
	$N_1 = K_1 K_3 K_5$	$K_1 = 3.85 \cdot 10^7$
	$N_2 = \frac{N_1}{K_{eq}}$	$K_2 = 8.388 \cdot 10^5$
	$D_1 = K_5 (K_3 + K_2)$	$K_3 = 6.84 \cdot 10^6$
	$D_2 = K_1 (K_3 + K_5)$	$K_4 = 4.032 \cdot 10^7$
	$D_3 = K_2 K_4$	$K_5 = 2.52 \cdot 10^5$
	$D_4 = K_6 (K_3 + K_2)$	$K_6 = 23.076 \cdot 10^6$
	$D_5 = K_1 K_4$	
	$D_6 = K_4 K_6$	

cients for the synthesis and degradation rates are given by ν_{ij} and ν_{ik}, respectively.

The number of concentration variables in the red cell mode, n, is 33 (Table 1) and the number of reaction rates, m, is 41 (Table 2). The kinetic rate expression for the 41 enzymatic reactions in glycolysis, 23DPG bypass, equilibrium reactions, pentose pathway, glutathione metabolism, nucleotide synthesis, and membrane transport are given in Tables 3–10. These information about

TABLE 4

The equilibrium constants for the reversible reactions considered in this model [11]

The unit for a concentration is mM.

Enzyme	Equilibrium expression	Constant
PGI	$K_{PGI} = \frac{F6P}{G6P}$	$K_{PGI} = 0.41$
TPI	$K_{TPI} = \frac{DHAP}{GAP}$	$K_{TPI} = 22.0$
GAPDH	$K_{GAPDH} = \frac{1,3DPG\ NADH\ H}{P\ GAP\ NAD}$	$K_{GAPDH} = 1.1294 \cdot 10^{-6}$
PGK	$K_{PGK} = \frac{3PG\ ATP}{ADP\ 1,3DPG}$	$K_1 = 25$
	$K_{pgk} = K_1 + K_1 Mg^{0.46}$	$K_2 = 3575$
PGM	$K_{PGM} = \frac{3PG}{2PG}$	$K_{PGM} = 6.8$
EN	$K_{EN} = \frac{PEP}{2PG}$	$K_{EN} = 0.59$
LDH	$K_{LDH} = \frac{LAC\ NAD}{PYR\ NADH\ H}$	$K_{LDH} = 7.075 \cdot 10^5$
AdK	$K_{AdK} = \frac{ATP\ AMP}{ADP^2}$	$K_1 = 0.081$
	$K_{AdK} = \frac{0.23(Mg + K_1)(Mg + K_2)}{(Mg + K_2)^2}$	$K_2 = 0.81$
		$K_3 = 22.2$
PRM	$K_{PRM} = \frac{R5P}{R1P}$	$K_{PRM} = 13.30$
PNPase	$K_{PNPase} = \frac{HX\ R1P}{P\ INO}$	$K_{PNPase} = 0.09$

enzyme kinetics are described in detail in the individual papers cited and summarized in [10–12].

The kinetics of metabolic pathways are coupled to several constraints: cofactor conservation, osmotic balance, electroneutrality, hemoglobin binding, magnesium complexation, and pH sensitivity of metabolic enzymes. Thus, the complete kinetic description consists of a combination of set of differential-algebraic equations, which have to be solved simultaneously to represent the metabolic state of the cell.

2.2. *Cofactor balances*

The model assumes that the total amount of the cofactors of redox metabolism are conserved. The conservation equations for these cofactors can be written as:

$$N_T = NAD^+ + NADH \tag{2}$$

$$NP_T + NADP^+ + NADPH \tag{3}$$

$$G_T = 2GSSG + GSH \tag{4}$$

In these expressions, NADH and NADPH correspond to one stoichiometric redox of hydrogen equivalent of the system whereas GSH corresponds to 1/2 hydrogen equivalent only.

2.3. *Membrane transport*

Membrane transport of glucose, adenine, water, sodium, potassium, pyruvate, lactate, hydrogen, chloride, and bicarbonate ions is considered in the red cell model (Fig. 2). Water has the highest transport rate, glucose and adenine transport is slower, chloride, bicarbonate, and hydrogen ions are even slower, and the time constants for the transport of sodium, potassium, pyruvate, and lactate ions are longer than those for other compounds. Only the transport of sodium, potassium, pyruvate, and lactate, is expressed in terms of rate equations (Table 10); all other transport is assumed to be at thermodynamic equilibrium across the cell membrane.

The function of chloride, bicarbonate, and hydrogen ions transport is to neutralize electrical charges inside and outside the cell, whereas water transport balances the osmotic pressure across the membrane. Glucose and adenine are taken up by the cell at such a fast rate that their intra- and extracellular concentrations are assumed to be the same. Sodium and potassium ions traverse the cell membrane via a sodium/potassium pump, and passive and facilitated fluxes. The pump utilizes metabolic energy, in term of ATP, to transport sodium and potassium ions against their concentration gradients across the membrane. On the other hand, the driving force for

TABLE 5

The rate expressions used to describe the kinetics of the reactions in the 2,3DPG bypass

The unit for a concentration is mM, and the unit for a reaction rate is mM/h. The references shown are for the pH dependence of kinetic constants. For the kinetic expressions, see [11].

Enzyme	Rate expression	Parameter values
DPG mutase (Werner, 1985)	$v = K_{DPGM}\dfrac{1,3DPG}{1+2,3DPG/K_{2,3DPG}}$	$K_{2,3DPG} = 0.04$
	$K_{DPGM} = k_0\, e^{-K_1+K_2PH}$, if pH > 6.85	$K_0 = 2.75\cdot10^5$
	$K_{DPGM} = 0$, if pH < 6.85	$K_1 = 19.367$
		$K_2 = 2.828$
2,3DPG phosphatase (Rapoport, 1977)	$v = \dfrac{V_m 2,3DPG}{K_{2,3DPG}+2,3DPG}$	$V_m = 0.52$
	$K_{2,3DPG} = 0.5028\, e^{-K_1+K_2PH}$	$K_1 = 8.0287$
		$K_2 = 0.98217$

TABLE 6

The rate expressions used to describe the kinetics of the oxidative reactions in the pentose pathway

The unit for a concentration is mM, and the unit for a reaction rate is mM/h. The references shown are for the pH dependence of kinetic constants. Kinetic expressions are given in [11].

Enzyme	Rate expression	Parameter values
G6P dehydrogenase		
(Soldin, 1968)	$v = \frac{E_t}{D}(N_1 AB - N_2 PQ)$	$E_t = 93 \cdot 10^{-9}$
	$D = D_1 + (D_2\mathrm{A} + D_3\mathrm{B} + D_4\mathrm{P} + D_5\mathrm{Q}) \cdot 10^{-3}$	$K = 3.867 \cdot 10^{-4}$
	$+ (D_6\mathrm{AB} + D_7\mathrm{AP} + D_8\mathrm{BQ} + D_9\mathrm{PQ}) \cdot 10^{-6}$	$K_1 = 1.1 \cdot 10^{8}$
	$+ (D_{10}\mathrm{ABP} + D_{11}\mathrm{BPQ}) \cdot 10^{-9}$	$K_2 = 0.87 \cdot 10^{3}$
	where A = NADP; B = G6P; P = GL6P; Q = NADPH	$K_3 = 0.26 \cdot 10^{8}$
	$N_1 = K_1 K_3 K_5 K_7 K_9 \frac{4187.16 \cdot 10^{-3}}{1 + \frac{\mathrm{H}}{K}}$	$K_4 = 0.30 \cdot 10^{3}$
	$N_2 = K_2 K_4 K_6 K_8 K_{10} \frac{4187.16 \cdot 10^{-3}}{1 + \frac{\mathrm{H}}{K}}$	$K_5 = 0.75 \cdot 10^{3}$
	$D_1 = K_2 K_9 (K_4 K_6 + K_4 K_7 + K_5 K_7)$	$K_6 = 2 \cdot 10^{3}$
	$D_2 = K_1 K_9 (K_4 K_6 + K_4 K_7 + K_5 K_7)$	$K_7 = 220 \cdot 10^{3}$
	$D_3 = K_3 K_5 K_7 K_9$	$K_8 = 11 \cdot 10^{8}$
	$D_4 = K_2 K_4 K_6 K_8$	$K_9 = 10 \cdot 10^{3}$
	$D_5 = K_2 K_{10} (K_4 K_6 + K_4 K_7 + K_5 K_7)$	$K_{10} = 14 \cdot 10^{8}$
	$D_6 = K_1 K_3 (K_5 K_7 + K_5 K_9 + K_6 K_9 + K_7 K_9)$	
	$D_7 = K_1 K_4 K_6 K_8$	
	$D_8 = K_3 K_5 K_7 K_{10}$	
	$D_9 = K_8 K_{10} (K_2 K_4 + K_2 K_5 + K_2 K_6 + K_4 K_6)$	
	$D_{10} = K_1 K_2 K_8 (K_5 + K_6)$	
	$D_{11} = K_3 K_8 K_{10} (K_5 + K_6)$	
6-Phosphoglucono-lactonase	$v = \frac{V_m \mathrm{GL6P}}{K_{\mathrm{GL6P}} + \mathrm{GL6P}}$	$V_M = 9.93$
		$K_{\mathrm{GL6P}} = 0.08$
GL6P dehydrogenase (Pearse, 1974)	$v = \frac{E_t}{D}(N_1 \mathrm{AB} - N_2 \mathrm{PQR})$	$E_t = 2.1 \cdot 10^{-6}$
		$K_a = 160.84$
	$D = D_1 + (D_2\mathrm{A} + D_3\mathrm{B} + D_4\mathrm{P} + D_5\mathrm{R}) \cdot 10^{-3}$	
	$+ (D_6\mathrm{AB} + D_7\mathrm{AP} + D_8\mathrm{BR} + D_9\mathrm{PQ})$	$K_b = 39.173$
	$+ (D_{10}\mathrm{P}_R + D_{11}\mathrm{QR}) \cdot 10^{-6}$	$K_c = 4.1322$
	$+ (D_{12}\mathrm{ABP} + D_{13}\mathrm{ABQ} + D_{14}\mathrm{APQ})$	$K_1 = 1.2 \cdot 10^{6}$
	$+ (D_{15}\mathrm{BQR} + D_{16}\mathrm{PQR}) \cdot 10^{-9}$	$K_2 = 4.1 \cdot 10^{2}$
	$+ (D_{17}\mathrm{ABPQ} + D_{18}\mathrm{BPQR}) \cdot 10^{-12}$	$K_3 = 1 \cdot 10^{9}$
	where A = NADP; B = GL6P; P = CO_2; Q = R5P; R = NADPH	$K_4 = 2.6 \cdot 10^{4}$
	$N_1 = K_1 K_3 K_5 K_7 K_9 K_{11} 38.7065 \cdot 10^{-3} (K_a - K_b H + K_c H^2)$	$K_5 = 0.48 \cdot 10^{2}$
	$N_2 = K_2 K_4 K_6 K_8 K_{10} K_{12} 38.7065 \cdot 10^{-6} (K_a - K_b H + K_c H^2)$	$K_6 = 0.30 \cdot 10^{2}$
	$D_1 = K_2 K_9 K_{11} (K_4 K_6 + K_4 K_7 + K_5 K_7)$	$K_7 = 6.3 \cdot 10^{2}$
	$D_2 = K_1 K_9 K_{11} (K_4 K_6 + K_4 K_7 + K_5 K_7)$	$K_8 = 0.036 \cdot 10^{6}$
	$D_3 = K_3 K_5 K_7 K_9 K_{11}$	$K_9 = 8.0 \cdot 10^{2}$
	$D_4 = K_2 K_4 K_6 K_8 K_{11}$	$K_{10} = 0.45 \cdot 10^{6}$
	$D_5 = K_2 K_9 K_{12} (K_4 K_6 + K_4 K_7 + K_5 K_7)$	$K_{11} = 3 \cdot 10^{2}$
	$D_6 = K_1 K_3 (K_5 K_9 K_{11} + K_6 K_9 K_{11} + K_7 K_9 K_{11}$	
	$+ K_5 K_7 K_9 + K_5 K_7 K_{11})$	$K_{12} = 9.9 \cdot 10^{6}$
	$D_7 = K_1 K_4 K_6 K_8 K_{11}$	
	$D_8 = K_3 K_5 K_7 K_9 K_{12}$	
	$D_9 = K_2 K_4 K_6 K_8 K_{10}$	

TABLE 6 (continued)

Enzyme	Rate expression	Parameter values
	$D_{10} = K_2K_4K_6K_8K_{12}$	
	$D_{11} = K_2K_{10}K_{12}(K_4K_6 + K_4K_7 + K_5K_7)$	
	$D_{12} = K_1K_3K_8K_{11}(K_5 + K_6)$	
	$D_{13} = K_1K_3K_5K_7K_{10}$	
	$D_{14} = K_1K_4K_6K_8K_{10}$	
	$D_{15} = K_3K_5K_7K_{10}K_{12}$	
	$D_{16} = K_8K_{10}K_{12}(K_2K_4 + K_2K_5 + K_2K_6 + K_4K_6)$	
	$D_{17} = K_1K_3K_8K_{10}(K_5 + K_6)$	
	$D_{18} = K_3K_8K_{10}K_{12}(K_5 + K_6)$	

the passive and facilitated fluxes is the concentration gradient. The steady state concentrations of both ions are the result of a balance between the pump, and the passive and facilitated fluxes.

2.4. Osmotic balance

Osmotic pressure is balanced across the membrane according to the following equations [10]:

$$\Pi_i = \Pi_e \tag{5}$$

and

$$\Pi_i = RT\sum_j \phi_{ij}C_{ij} \tag{6}$$

$$\Pi_e = RT\sum_j \phi_{ej}C_{ej} \tag{7}$$

where R is the ideal gas constant, T denotes the temperature, ϕ represents the osmotic coefficient, and C_j is the concentration of component j. The osmotic pressure balance is satisfied by the fast water transport through the membrane. The result of the balance also determines the cell volume.

The intracellular osmolarity can be partitioned into 4 terms according to the membrane permeability of substrates and the volume-dependence of substrate concentrations:

$$\Pi_i = \Pi_1 + \Pi_2 + \Pi_3 + \Pi_{Hb} \tag{8}$$

where:

- Π_1 is contributed by slow membrane permeable metabolites and cations, such as G6P, FDP, ATP, and Na, of which the rates of reaction are expressed in term of ordinary differential equations in the simulator. The concentrations of substrates included in Π_1 vary with the cell volume, since they have relatively small membrane permeability.
- Π_2 is contributed by relatively fast membrane permeable substrates, such as glucose and adenine, of which the intracellular concentrations can be assumed to be at equilibrium with the extracellular concentrations. Therefore, the magnitude of Π_2 is not a function of cell volume, when the external nutrient concentrations are constant.
- Π_3 is contributed by anions, such as Cl^- and CO_3^-, which transport instantaneously through the membrane to balance cation transport.
- Π_{Hb} is contributed by hemoglobin molecules. The changes in the osmotic coefficient of hemoglobin ϕ_{Hb} as a function of its concentration is given by [13].

$$\phi_{Hb} = 1.0 + 0.0645Hb + 0.0258Hb^2 \tag{9}$$

where the concentration of hemoglobin varies with the red cell volume v as $Hb = Hb^0/V$.

2.5. Electroneutrality

The law of electrical neutrality of solutions applies within and outside the erythrocyte. There

TABLE 7

The rate expressions used to describe the kinetics of the non-oxidative reactions in the pentose pathway

The unit for a concentration is mM, and the unit for a reaction rate is mM/h. The references shown are for the pH dependence of kinetic constants. Kinetic expressions are given in [19].

Enzyme	Rate expression	Parameter values
R5PI	$v = \frac{V_f(RU5P - R5P/K_{eq})}{K_1 + RU5P + K_1/K_2 R5P}$	$V_f = 1\,702.296$ $K_{eq} = 2.57$ $K_1 = 0.78$ $K_2 = 2.2$
XU5PE	$v = \frac{V_f(RU5P - X5P/K_{eq})}{K_1 + RU5P + K_1/K_2 \times 5P}$	$V_f = 4\,633.56$ $K_{eq} = 3.0$ $K_1 = 0.19$ $K_2 = 1.5$
TK1	$\frac{(N_1 A - N_2 PQ)ET}{D_1 A + D_2 B + D_3 P + D_4 Q + D_5 AB + D_6 PQ + D_7 BQ + D_8 AP}$ where A = X5P; B = R5P; P = GAP; Q = S7P $N_1 = K_1 K_3 K_5 K_7$ $N_2 = \frac{N_1}{K_{eq}}$ $D_1 = K_1 K_3 (K_6 + K_7)$ $D_2 = K_5 K_7 (K_2 + K_3)$ $D_3 = K_2 K_4 (K_6 + K_7)$ $D_4 = K_6 K_8 (K_2 + K_3)$ $D_5 = K_1 K_5 (K_3 + K_7)$ $D_6 = K_4 K_8 (K_2 + K_6)$ $D_7 = K_5 K_8 (K_2 + K_3)$ $D_8 = K_1 K_4 (K_6 + K_7)$	$K_{eq} = 1.2$ $ET = 0.33 \cdot 10^{-3}$ $K_1 = 7.776 \cdot 10^5$ $K_2 = 1.368 \cdot 10^5$ $K_3 = 1.224 \cdot 10^5$ $K_5 = 5.616 \cdot 10^5$ $K_5 = 11.844 \cdot 10^5$ $K_6 = 6.3 \cdot 10^5$ $K_7 = 1.44 \cdot 10^5$ $K_8 = 16.128 \cdot 10^4$
TK2	$\frac{(N_1 A - N_2 PQ)ET}{D_1 A + D_2 B + D_3 P + D_4 Q + D_5 Ab + D_6 PQ + D_7 BQ + D_8 AP}$ where A = X5P; B = E4P; P = GAP; Q = F6P $N_1 = K_1 K_3 K_5 K_7$ $N_2 = \frac{N_1}{K_{eq}}$ $D_1 = K_1 K_3 (K_6 + K_7)$ $D_2 = K_5 K_7 (K_2 + K_3)$ $D_3 = K_2 K_4 (K_6 + K_7)$ $D_4 = K_6 K_8 (K_2 + K_3)$ $D_5 = K_1 K_5 (K_3 + K_7)$ $D_6 = K_4 K_8 (K_2 + K_6)$ $D_7 = K_5 K_8 (K_2 + K_3)$ $D_8 = K_1 K_4 (K_6 + K_7)$	$K_{eq} = 10.3$ $ET = 0.33 \cdot 10^{-3}$ $K_1 = 7.776 \cdot 10^5$ $K_2 = 1.368 \cdot 10^5$ $K_3 = 1.224 \cdot 10^5$ $K_4 = 5.616 \cdot 10^5$ $K_5 = 8.064 \cdot 10^5$ $K_6 = 6.3 \cdot 10^5$ $K_7 = 1.44 \cdot 10^5$ $K_8 = 7.668 \cdot 10^4$
TA	$\frac{(N_1 A - N_2 PQ)ET}{D_1 A + D_2 B + D_3 P + D_4 Q + D_5 AB + D_6 PQ + D_7 BQ + D_8 AP}$ where A = S7P; B = GAP; P = E4P; Q = F6P $N_1 = K_1 K_3 K_5 K_7$ $N_2 = \frac{N_1}{K_{eq}}$	$K_{eq} = 1.05$ $ET = 0.69 \cdot 10^{-3}$ $K_1 = 20.88 \cdot 10^5$ $K_2 = 1.6308 \cdot 10^5$

TABLE 7 (continued)

Enzyme	Rate expression	Parameter values
TA	$D_1 = K_1K_3\,(K_6 + K_7)$	$K_3 = 5.868 \cdot 10^4$
	$D_2 = K_5K_7\,(K_2 + K_3)$	$K_4 = 3.636 \cdot 10^6$
	$D_3 = K_2K_4\,(K_6 + K_7)$	$K_5 = 17.64 \cdot 10^5$
	$D_4 = K_6K_8\,(K_2 + K_3)$	$K_6 = 2.16 \cdot 10^5$
	$D_5 = K_1K_5\,(K_3 + K_7)$	$K_7 = 6.12 \cdot 10^4$
	$D_6 = K_4K_8\,(K_2 + K_6)$	$K_8 = 28.44 \cdot 10^4$
	$D_7 = K_5K_8\,(K_2 + K_3)$	
	$D_8 = K_1K_4\,(K_6 + K_7)$	

TABLE 8

The rate expressions used to describe the kinetics of the reactions of glutathione metabolism

The unit for a concentration is mM, and the unit for a reaction rate is mM/h. The references shown are for the pH dependence of kinetic constants. Kinetic expressions are given in [11].

Enzyme	Rate expression	Parameter value
GSSG reductase (NADPH)		$E_t = 125 \cdot 10^{-9}$
(Scott, 1963)	$v = \frac{E_t}{D}(N_1\mathrm{AB} - N_2\mathrm{P}^2\mathrm{Q})$	$K_a = -2880$
	$D = D_1 + (D_2\mathrm{A} + D_3\mathrm{B} + D_4\mathrm{P} + D_5\mathrm{Q}) \cdot 10^{-3}$	$K_b = 902.33$
	$+ [D_6\mathrm{AB} + D_7\mathrm{AP} + D_8\mathrm{BQ}$	$K_c = -65.272$
	$+ D_9\mathrm{P}^2 + (D_{10} + D_{11})\mathrm{PQ}] \cdot 10^{-6}$	$K_1 = 0.85 \cdot 10^8$
	$+ [(D_{12} + D_{13})\mathrm{ABP} + D_{14}\mathrm{AP}^2$	$K_2 = 0.51 \cdot 10^3$
	$+ D_{15}\mathrm{BPQ} + D_{16}\mathrm{P}^2\mathrm{Q}] \cdot 10^{-9}$	$K_3 = 1 \cdot 10^8$
	$+ (D_{17}\mathrm{ABP}^2 + D_{18}\mathrm{BP}^2\mathrm{Q}) \cdot 10^{-12}$	$K_4 = 7.2 \cdot 10^3$
	where A = NADP, B = GSSG, P = GSH, Q = NADP	$K_5 = 0.81 \cdot 10^3$
	$N_1 = K_1K_3K_5K_7K_9K_{11}\ 15\,455.6\,(K_a + K_b pH + K_c pH^2)$	$K_6 = 1.0 \cdot 10^3$
	$N_2 = K_2K_4K_6K_8K_{10}K_{12}\ 15\,455.6\,(K_a + K_b pH + K_c pH^2)$	$K_7 = 1.0 \cdot 10^6$
	expressions for Di's are the same as those	$K_8 = 0.5 \cdot 10^8$
	for GL6P dehydrogenase	$K_9 = 1.0 \cdot 10^6$
		$K_{10} = 0.5 \cdot 10^8$
		$K_{11} = 7.0 \cdot 10^3$
		$K_{12} = 1 \cdot 10^8$
GSSG reductase (NADH)		
(Scott, 1963)	$v = \frac{V_\mathrm{m}}{1 + \frac{K_{md}(1 + K_{ig}\mathrm{GSSG})}{\mathrm{NADH}} + \frac{K_{mg}(1 + K_{id}\mathrm{NADH})}{\mathrm{GSSG}}}$	$K_{md} = 0.193$
	$V_\mathrm{m} = 0.041\ 10^{-0.66276 + 0.3991\mathrm{PH}}$ when pH < 6.12	$K_{mg} = 0.0167$
	$V_\mathrm{m} = 0.041\ 10^{4.1304 - 0.38221PH}$ when pH > 6.12	$K_{id} = 1.5$
		$K_{ig} = 0.141$
GSH turnover		
	$v = k\ \mathrm{GSH}$	$K_1 = -2880$
	$k = 1.1429 \cdot 10^{-3}\,(K_1 + K_2\mathrm{pH} + K_3\mathrm{pH}^2)$	$K_2 = 902.33$
		$K_3 = -65.272$

TABLE 9

The rate expressions used to describe the kinetics of the reactions involved in nucleotide synthesis

The unit for a concentration is mM, and the unit for a reaction rate is mM/h. The references shown are for the pH dependence of kinetic constants. Kinetic expressions are given in [11].

Enzyme	Rate expression	Parameter values
AMPase (Heppel, 1951)	$v = k\ \mathrm{AMP}$ $k = \dfrac{1.6480}{1 + \dfrac{\mathrm{H}}{K_1} + \dfrac{K_2}{\mathrm{H}}}$	$K_1 = 2.5642 \cdot 10^{-3}$ $K_2 = 1.2159 \cdot 10^{-6}$
Adenosine deaminase (Daddona, 1977)	$v = \dfrac{V_M \mathrm{A}}{K + \mathrm{A}}$ $V_m = \dfrac{36.348}{1 + \dfrac{\mathrm{H}}{K_1} + \dfrac{K_2}{\mathrm{H}}}$	$K = 0.052$ $K_1 = 1.995 \cdot 10^{-4}$ $K_2 = 3.162 \cdot 10^{-5}$
Adenosine kinase (Meyskens, 1971)	$v = V_m \dfrac{\mathrm{ATP}}{K_{\mathrm{ATP}} + \mathrm{ATP}} \dfrac{\mathrm{A}}{K_A + \mathrm{A}}$ $V_m = \dfrac{8.6229}{1 + \dfrac{K_1}{\mathrm{H}}}$	$K_{\mathrm{ATP}} = 0.8$ $K_A = 0.0004$ $K_1 = 1.636 \cdot 10^{-4}$
AMP deaminase	$v = V_m \dfrac{\mathrm{AMP}}{K + \mathrm{AMP}}$	$V_{\mathrm{M}} = 0.01$ $K = 0.8$
ATPase (Dunham, 1961)	$v = K_{\mathrm{ATPase}} \mathrm{ATP}$ $K_{\mathrm{ATPase}} = \dfrac{0.415}{1 + \dfrac{\mathrm{H}}{K_1} + \dfrac{K_2}{\mathrm{H}}}$	$K_1 = 3.7342 \cdot 10^{-3}$ $K_2 = 8.2714 \cdot 10^{-7}$
AdPRT (Thomas, 1973)	$v = V_m = \dfrac{\mathrm{ADE}}{K_{\mathrm{ADE}} + \mathrm{ADE}} \dfrac{\mathrm{PRPP}}{K_{\mathrm{PRPP}} + \mathrm{PRPP}}$ $V_m = \dfrac{0.202}{1 + \dfrac{\mathrm{H}}{K_1} + \dfrac{K_2}{\mathrm{H}}}$	$K_{\mathrm{ADE}} = 0.0023$ $K_{\mathrm{PRPP}} = 0.0195$ $K_1 = 3.981 \cdot 10^{-5}$ $K_2 = 3.162 \cdot 10^{-7}$
IMPase (Heppel, 1951)	$v = \dfrac{K_{\mathrm{IMP}}}{1 + \mathrm{INO}/K_{\mathrm{INO}}}$ $K = \dfrac{0.09387}{1 + \dfrac{\mathrm{H}}{K_1} + \dfrac{K_2}{\mathrm{H}}}$	$K_1 = 2.5642 \cdot 10^{-3}$ $K_2 = 1.2159 \cdot 10^{-6}$ $K_{\mathrm{INO}} = 25.0$

TABLE 9 (continued)

Enzyme	Rate expression	Parameter values
PRPP synthethase	$V = V_m V_r \dfrac{\text{R5P ATP} - \text{PRPP AMP}/K_{eq}}{N}$	$K_{ATP} = 0.17$
(DeVerdier, 1963)	$V_m = \dfrac{K_0}{1 + \dfrac{H}{K_1} + \dfrac{K_2}{H}}$	$K_{R5P} = 0.65$
	$N = V_r K_{R5P}\text{ATP} + V_r K_{ATP}\text{R5P} + V_m K_{AMP}\text{PRPP}/K_{eq} + V_m K_{PRPP}\text{AMP}/K_{eq} + V_r\text{R5P ATP} + V_m\text{PRPP AMP}/K_{eq}$	$K_{eq} = 28.6$
	$V_r = V_m / K_v$	$K_v = 7.5$ $K_{PRPP} = 0.09$ $K_{AMP} = 0.275$ $K_0 = 0.836$ $K_1 = 1.0099 \cdot 10^{-4}$ $K_2 = 1.8835 \cdot 10^{-6}$
HGPRT	$v = V_m \dfrac{\text{PRPP}}{K_{PRPP} + \text{PRPP}} \dfrac{\text{HX}}{K_{HX} + \text{HX}}$	$V_m - 0.2011$ $K_{PRPP} = 0.005$ $K_{HX} = 0.22$

must be the same number of positive ionic charges in solution as there are negative charges.

$$\sum_{j=1}^{n} z_{ij} C_{ij} = 0 \tag{10}$$

$$\sum_{j=1}^{n} z_{ej} C_{ej} = 0 \tag{11}$$

where z_{ij} and z_{ej} represent the charges on intracellular and extracellular ions. With physiological ATP level, H^+/Cl^- and H^+/HCO_3^- ion pairs transport is much faster than other ion pairs [14,15]. The instantaneous cell volume change due to pH variation is then a result of fast HCl and H_2CO_3 transport. The intracellular H^+ concentration is buffered mostly by hemoglobin molecular, of which the dissociable functional groups concentration is the most abundant among all molecules in the red cell. Therefore, the primary source of intracellular hydrogen ions is hemoglobin molecules. Accordingly, two reactions are involved in the volume variation process when the cell is subjected to different pH media: the diassociation of hemoglobin and the cotransport of hydrogen ion and anions.

$$H - Hb \rightleftharpoons Hb^- + H_{in}^+ \tag{12}$$

$$H_{in}^+ + An_{in}^- \rightleftharpoons H_{ex}^+ + An_{ex}^- \tag{13}$$

Instantaneous cotransport of H^+ and anions is assumed, and an equilibrium expression for the reversible reactions, 12 and 13, can be written as follows:

$$\frac{(Hb + x)H_e An_e}{(HHb - x)(An_i - y)} = K_e e^{-ZF\Delta\Psi/RT} \tag{14}$$

where x is the moles of hemoglobin disassociated after a perturbation, y is the number of H^+/anions pairs transported through the membrane after the perturbation, R is the ideal gas constant, T is the temperature, K_e is the disassociation constant of the functional group on hemoglobin molecule, Z is the charge on the ions, F is the faraday constant, $\Delta\Psi$ is the membrane potential,

subscripts i and e represent the initial intra- and extra-cellular conditions. It can be assumed that x is approxmately equal to y since $[An_i] \gg [H_i]$, and can be solved by Eqn. 14.

2.6. Mg complexation

Free Mg forms complexes with ATP, ADP, AMP, 23DPG, and their hydrogenated molecules.

TABLE 10

The rate expressions used to describe the kinetics of membrane transport processes considered in this model [11]

The unit for a concentration is mM, and the unit for a reaction rate is mM/h. The references shown are for the pH dependence of kinetic constants.

Transport	Expression	Parameter values
Passive Na flux		
(Harris, 1954)	$v = K_x ln(r)\dfrac{Na_e - rNa_i}{r-1} + V_m\left(\dfrac{Na_e}{K_m + Na_e} - \dfrac{rNa_i}{K_m + rNa_i}\right)$	
	$V_m = 2.816\,(-1.16+0.3\,PH)$	$K_m = 21.0$
	$K_x = 7.055 \cdot 10^{-3}\,(-1.16+0.3\,PH)$	
Passive K flux		
(Harris, 1954)	$v = K_x ln(r)\dfrac{K_e - rK_i}{r-1} + V_m\left(\dfrac{K_e}{K_m + K_e} - \dfrac{rK_i}{K_m + rK_i}\right)$	
	$V_m = 3.115\,(-1.16+0.3\,PH)$	$K_m = 4.0$
	$K_x = 6.349 \cdot 10^{-3}\,(-1.16+0.3\,PH)$	
Na/K pump		
(Dunham, 1966)	$v = \dfrac{ATP}{K_{ATP} + ATP} \dfrac{\frac{V_m}{2}K_e^2 + B_2 K_e}{B_1 B_2 + 2B_2 K_e + K_e^2 + \left(\frac{B_3}{Na_i} + 1\right)^3 \left(B_1 B_2 \frac{K_2}{K_1} + \frac{K_3}{K_1}\left(K_e^2 + eB_2 K_e\right)\right)}$	
	$V_m = \dfrac{3,4}{1 + \frac{H}{K_a} + \frac{K_b}{H}}$	$K_{ATP} = 0.764$
		$B_1 = 0.0617$
		$B_2 = 0.1328$
		$B_3 = 6.2672$
		$\dfrac{K_2}{K_1} = 0.0082$
		$\dfrac{K_3}{K_1} = 0.0501$
		$e = 0.7114\epsilon = 0.7114$
		$K_a = 3.7342 \cdot 10^{-3}$
		$K_b = 8.2714 \cdot 10^{-7}$
Pyruvate	$v = K\,(PYR - PYR_{ext})$	$K = 63.8$
Lactate	$v = K(LAC - LAC_{ext})$	$K = 12.8$
Hypoxanthine	$v = P_m HX + V_m \dfrac{HX}{HX + K_{HX}}$	$P_m = 37.8$
		$V_m = 151.6$
		$K_{HX} = 0.4$
Adenine	$v = V_m\left(\dfrac{ADE_i}{K_m + ADE_i} - \dfrac{ADE_e}{K_m + ADE_e}\right)$	$V_m = 90.0$
		$K_m = 2.6$

These Mg complexes regulate several important glycolytic enzymes, such as HK, PFK, and PK. Let AMP_T, ADP_T, ATP_T, and $2,3DPG_T$ denote the total amount of phosphorylated metabolites; AMP, ADP, ATP and 2,3DPG represent the total free amount of these metabolites; and MgAMP, MgADP, MgHADP, MgATP, MgHATP and Mg23DPG represent the corresponding magnesium complexes, then the equilibrium equations relating these concentration variables may be written as [16]:

$$\frac{[MgATP^{2-}]}{[Mg^{2+}][ATP^{4-}]} = k_{b,ATP} = 1.39 \cdot 10^4 M^{-1}$$

$$\frac{[MgHATP^{1-}]}{[Mg^{2+}][HATP^{3-}]} = k_{b,HATP} = 3.55 \cdot 10^1 M^{-1}$$

$$\frac{[MgADP^{-}]}{[Mg^{2+}][ADP^{3-}]} = k_{b,ADP} = 1.32 \cdot 10^3 M^{-1}$$

$$\frac{[MgHADP^{0}]}{[Mg^{2+}][HADP^{2-}]} = k_{b,HADP} = 3.24 \cdot 10^1 M^{-1}$$

$$\frac{[MgAMP^{0}]}{[Mg^{2+}][AMP^{2-}]} = k_{b,AMP} = 6.01 \cdot 10^1 M^{-1}$$

$$\frac{[Mg2,3DPG^{2-}]}{[Mg^{2+}][2,3DPG^{4-}]} = k_{b,2,3GDP} = 5.98 \cdot 10^2 M^{-1} \qquad (15)$$

The disassociation constants for ATP, ADP, and AMP are [16]:

$$\frac{[H^+][ATP^{4-}]}{[HATP^{3-}]} = k_{a,ATP} = 1.08 \cdot 10^{-7} M$$

$$\frac{[H^+][ADP^{3-}]}{[HADP^{2-}]} = k_{a,ADP} = 1.20 \cdot 10^{-7} M$$

$$\frac{[H^+][AMP^{2-}]}{[HAMP^{1-}]} = k_{a,AMP} = 3.24 \cdot 10^{-7} M \qquad (16)$$

The corresponding mass conservation equations are:

$$ATP_T = ATP + HATP + MgATP + MgHATP$$

$$ADP_T = ADP + HADP + MgADP + MgHADP$$

$$AMP_T = AMP + HAMP + MgAMP$$

$$2,3DPG_T = 2,3DPPG + Mg2,3DPG$$

$$Mg_T = Mg + MgAMP + MgADP + MgATP + Mg2,3DPG \qquad (17)$$

Eq. (15–17) are the 14 set of equations with fourteen unknowns, where the unknowns are the free and the magnesium complexed metabolites. If the equilibrium constants are known [8,16] and the total amount of adenosine phosphates and 2,3DPG is known (these variables are time dependent quantities in the dynamic model), this set of equations can be solved for the unknowns.

2.7. Hemoglobin binding

Hemoglobin molecule binds to ligands such as O_2, CO_2, Cl^-, 23DPG, and H^+. The binding affinity of a hemoglobin molecule to the ligands depends on the thermodynamic properties of the red cell plasma, e.g., the concentration of ligands.

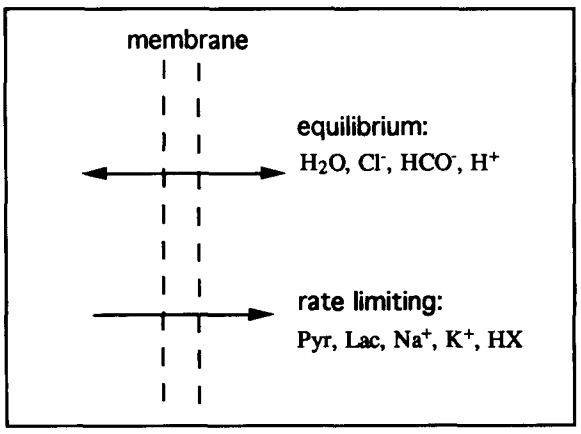


Fig. 2. Membrane transport processes can be separated into two groups: those which are fast and reach equilibrium; and those which are rate limiting.

A 'state' of hemoglobin molecule is a specification of all the binding sites, together with a specification of quaternary structure of the molecule (T or R). The possibility of occurrence of a 'state' of the hemoglobin is proportional to the probability weight W of the state. Given ligand concentrations, the partition function P is the sum of the probability weights of all possible states. The partition function for hemoglobin binding can then be expressed as [17]:

$$P = W_{H^+}(W_R + W_T) \tag{18}$$

where W_{H^+}, W_R, and W_T can be further factorized:

$$\begin{aligned} W_R = {} & \{1 + Q_{\text{roxy}}[O_2]\}^4 \\ & \times \left\{ 1 + Q_{\text{rbh1}}[H^+](1 + Q_{\text{rcl1}}[Cl^-]) \right. \\ & \left. + Q_{\text{rcdx}} \frac{[CO_2]}{[H^+]} \right\}^2 \\ & \times \left\{ \left[(1 + Q_{\beta\text{atr}}[H^+])^2 (Q_{\text{rbpg5}}[23\text{DPG}^{5-}] \right. \right. \\ & + Q_{\text{rbpg4}}[23\text{DPGH}^{4-}] + Q_{\text{rbpg3}}[23\text{DPGH}_2^{3-}] \\ & \left. + \left(1 + Q_{\beta\text{atr}}[H^+] + Q_{\text{rcdx}} \frac{[CO_2]}{[H^+]} \right)^2 \right\} \\ & \times \{1 + Q_{\text{rbh2}}[H^+]\}^4 \times \{1 + Q_{\text{rbh3}}[H^+]\}^4 \\ & \times \{1 + Q_{\text{rcl2}}[Cl^-]\}^2 \end{aligned} \tag{19}$$

$$\begin{aligned} W_T = {} & Q_{\text{allo}} \times \{1 + Q_{\text{toxy}}[O_2]\}^4 \\ & \times \left\{ 1 + Q_{\text{tbh1}}[H^+](1 + Q_{\text{tcl1}}[Cl^-]) \right. \\ & \left. + Q_{\text{tcdx}} \frac{[CO_2]}{[H^+]} \right\}^2 \\ & \times \left\{ \left[(1 + Q_{\beta\text{atr}}[H^+])^2 (Q_{\text{tbpg5}}[23\text{DPG}^{5-}] \right. \right. \\ & + Q_{\text{tbpg4}}[23\text{DPGH}^{4-}] \\ & + Q_{\text{tbpg3}}[23\text{DPGH}_2^{3-}] \\ & \left. + \left(1 + Q_{\beta\text{atr}}[H^+] + Q_{\text{tcdx}} \frac{[CO_2]}{[H^+]} \right)^2 \right\} \\ & \times \{1 + Q_{\text{tbh2}}[H^+]\}^4 \times \{1 + Q_{\text{tbh3}}[H^+]\}^4 \\ & \times \{1 + Q_{\text{tcl2}}[Cl^-]\}^2 \end{aligned} \tag{20}$$

$$\begin{aligned} W_{H^+} = {} & \{1 + Q_{\text{ctr}}[H^+]\}^4 \times \{1 + Q_{\text{hem}}[H^+]\}^8 \\ & \times \{1 + Q_{\text{glu}}[H^+]\}^{54} \times \{1 + Q_{\text{his}}[H^+]\}^{16} \\ & \times \{1 + Q_{\text{cys}}[H^+]\}^2 \times \{1 + Q_{\text{tyr}}[H^+]\}^8 \\ & \times \{1 + Q_{\text{lys}}[H^+]\}^{44} \times \{1 + Q_{\text{arg}}[H^+]\}^{12} \end{aligned} \tag{21}$$

The Qs are the effective association constants for the various binding reactions [17]. The occupancy number of each ligand, which is defined as the average number of the ligand molecules bound to a hemoglobin molecule, can be calculated from Eqn. 18 as follows:

$$Y_l = \frac{d\ln P}{d\ln[l]} = \frac{[l]}{P} \frac{dP}{d[l]} \tag{22}$$

where Y_l is the occupancy number of ligand, l, and can be easily calculated numerically or analytically by taking the derivative of Eqn. 18.

3. Program structure

The ordinary differential equations and the algebraic equations describing the red blood cell, Eqns. 1–17, are solved using the ordinary differential equation integrator DEPISODE. This li-

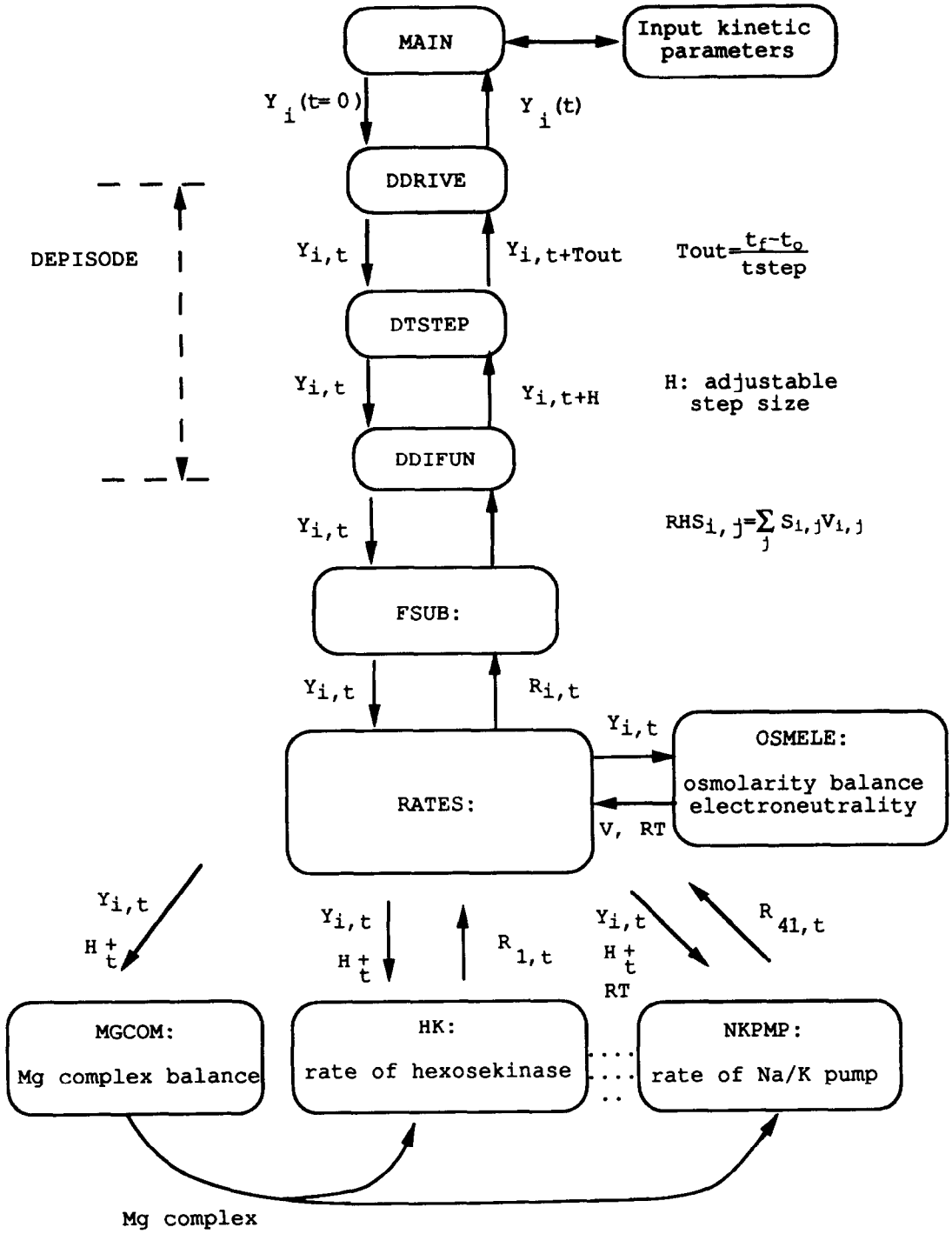


Fig. 3. The flow diagram of the computer simulator for human red blood cell.

brary requires a user supplied subroutine, named FSUB, which receives the values of the time dependent variables at a particular point in time and calculates the time derivatives of the variables at that instant; i.e.,

$$y \text{ at time } t \xrightarrow{\text{FSUB}} \frac{dy}{dt} \text{at time } t \tag{23}$$

Subroutine FSUB must thus calculate the reaction rate of each metabolic reaction given the metabolite concentrations, under the algebraic constraints imposed.

Since the time derivative are evaluated from

$$\frac{dy}{dt} = S \cdot v(y)V \tag{24}$$

where y corresponds to VC in Eqn. 1 and S is the stoichiometric matrix. One must evaluate the reaction rates from the concentration vector y that is supplied. The subroutine FSUB calls a subroutine RATES, that calculates the metabolic reaction rates given the metabolite concentrations, before carrying out the matrix multiplication specified in Eqn. 24. RATES then in turn calls the subroutines for individual metabolic reaction rates. The parameters for each reaction rate kinetics can be altered through a menu driven format.

The reaction rate laws require concentration variables that are not any of the dynamic variables *ys*. These concentrations are calculated through the coupled algebraic equations. In addition, the electroneutrality and osmotic balance equations must be balanced by assigning the appropriate values to volume and Donnan ratio.

The sequence in which the calculations are carried out is; first, electroneutrality and osmolarity balance are satisfied by OSMELE, then magnesium complexes concentrations are evaluated by MGCOM, followed by the computation of individual reaction rates. Notice that all the fast processes are calculated before metabolic reaction rates because the formers are assumed to be at equilibrium at all times. Therefore, in each iteration of FSUB and RATES, all the three fast processes-electroneutrality, osmolarity balance, and magnesium complexation-will be satisfied before the slow metabolic reaction rates are calculated.

The flow diagram of the computer program is shown in Fig. 3. All the subroutines between the MAIN program and subroutine DDIFUN belong to the ODE solver DEPISODE, which integrates dependent variables Y_i given derivatives RHS_i. The rest of the program takes Y_i, calculates RHS_i, and return the derivatives to DEPISODE.

4. Software package description

The initial screen display of the Macintosh software package consists of a main menu and a diagram of the metabolic pathways of the red cells. The titles in the menu include 'command', 'glycolysis', 'pentose', 'nucleotide', 'steady', and 'integration'. The users can examine each enzyme kinetic equation and change parameter values in the kinetics by either choosing the enzyme under menu titles 'glycolysis', 'pentose', and 'nucleotide', or by clicking the enzyme in the diagram of the metabolic pathways. Either way, the kinetic equations for the selected enzyme will show up in a new window, and one can decide to vary the kinetic parameters by reading them from a data file. To read the data file storing the new parameter values, the user can select item 'read file' under menu title 'read parameter'. The name of the data file must not contain any space and symbol except period '.'. In the data file the parameter values must be in the same sequence as those appeared on the screen, and a 'return' key must be used after the last data entry to serve as the 'end of file' command. To go back to the main menu after viewing kinetic equations, the user can select item 'done' under menu title 'command'.

The user can choose to calculate steady state metabolite concentrations by selecting item 'steady state' under title 'steady' from the main menu. When calculating the steady state solution, the package uses the Newton's routine and reads the initial guess from the default data file 'ss.dat'. The data in 'ss.dat' are in the following order: number of equations, the concentrations of 33

metabolites listed in Table 1, the accuracy of the calculation, and the allowable maximum iteration for the Newton's method. The user can input new initial guess from another data file by selecting menu item 'read guess values' under the same menu title. To calculate the steady state solution, simply select item 'steady state', where the steady state solution will be calculated and saved in the text file 'steady-filename', where 'filename' is the file containing the guess values ('ss.dat' in default). The format of the steady state solution output file is the 33 metabolite concentrations in the same order as in Table 1.

To determine the response of the red cell metabolism to a perturbation, the user can select item 'integrate' under menu title 'integration' of the main menu. The perturbation can be specified in data files 'integrate.dat' and 'initial.dat'. The data in 'integrate.dat' are: the number of metabolites, the concentrations of the metabolites listed in Table 1, the initial time, the final time of integration, the number of outputs between the initial and final time, and the relative accuracy of the calculation. The initial values of metabolite concentrations for integration can also be read interactively by choosing menu item 'read initial value' from menu title 'integration'. The data in 'initial.dat' are: osmolarity, concentrations of glucose, adenine, carbon dioxide, total glutathione, extracellular lactate, total magnesium, total NADH, P_i, PP_i, extracellular pyruvate, hemoglobin, and extracellular pH. The integrated metabolite concentrations are saved in file 'concentration-filename', and the metabolite fluxes are saved in file 'flux-filename', where 'filename' is the file containing the initial conditions for integration. The output files contain the integration results at various time. It shows the number of printout, the time for integration, and 33 concentrations in Table 1, 41 fluxes in Table 2, cell volume, Donnan ratio, Mg complexes, and hemoglobin binding coefficience. After completing the integration, metabolite concentrations or fluxes can be plotted against time by selecting items 'concentration' or 'flux' under the same menu title. If the formats of integration output files 'cmpd.out' and 'rates.out' are incorrected or altered for some reasons, errors will occur while the simulator is reading the files before plotting. The output files of integration, 'flux-filename' and 'concentration-filename', can also be read by commercial plotting packages such as Cricket Graph™. When using a plotting routine, the user should delete the first row of each output file, which is the variable names, and specify the data format as numerical instead of alphabetic.

The response of the red cell metabolism can be calculated with the external pH, osmolarity glucose, adenine, phosphate, and lactate concentrations as functions of time. Two types of time-dependent functions are available in the menu 'integration', linear and step wave. The user can choose the desired time-dependent perturbation of external parameters, and key in the slope and interception for the linear functions, or period and altitude for the step wave functions. The calculation time may vary among cases, depending on the time variation functions. A large change of external parameter creates a stiff numerical problem, and it will take a longer time to integrate such a problem, especially around the discontinuous points of the step wave functions. The user may want to be careful not to give the perturbation functions negative values or unreasonable deviation from the physiological conditions. The stage of integration will be indicated on the screen, and the user can stop the calculation by clicking the 'cancel' box several times.

The user can also examine each enzyme activity as functions of several parameters by selecting item 'enzyme vs parameter' under menu title 'command' from the main menu. Once the item is selected, a new set of menu appears. The user must first choose one parameter under menu title 'parameter', and one enzyme under menu titles, 'fast process', 'glycolysis', 'pentose', or 'nucleotide', before selecting 'calculate response' under 'commands' to calculate the chosen enzyme activity as a function of the chosen parameter. The results that show the activity or corresponding metabolite concentrations of the selected enzyme as function of the selected parameter will be plotted on the screen.

The hemoglobin binding curves can be calculated and plotted as functions of ligand concentrations by selecting menu item 'hemoglobin

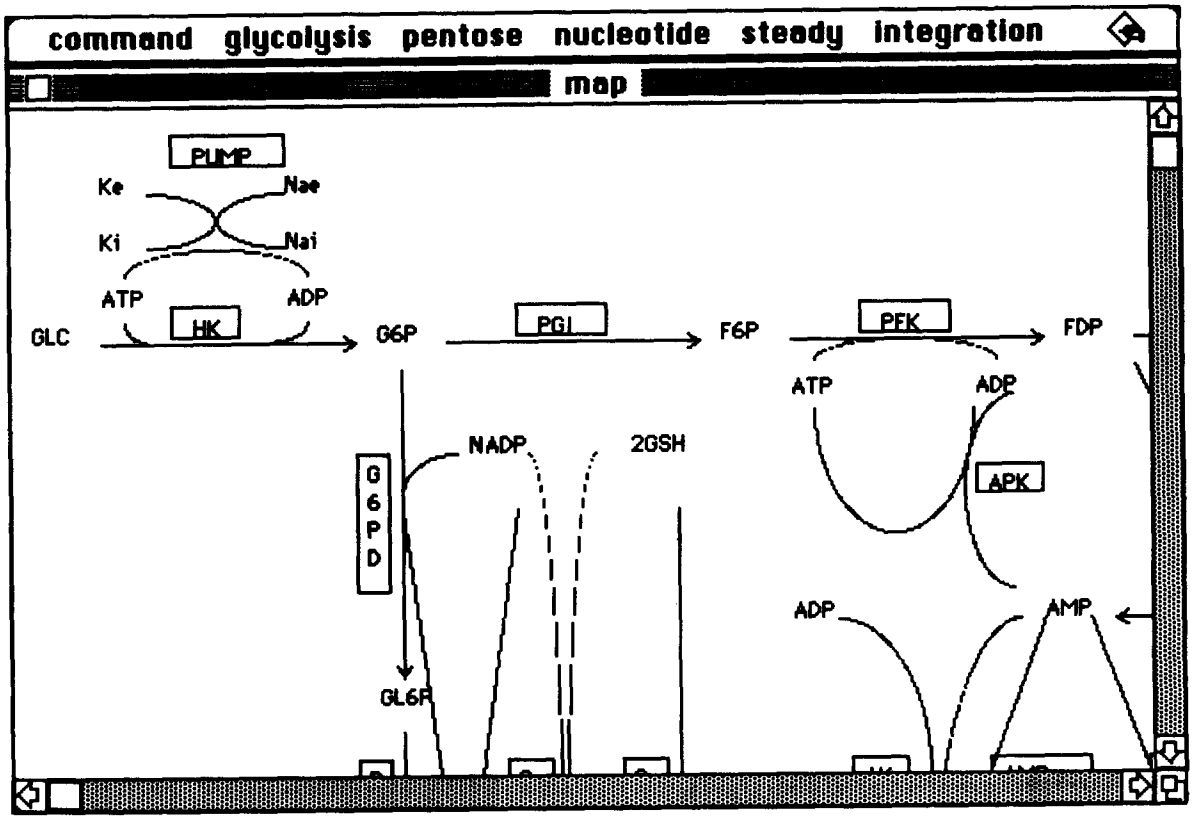
binding' under menu title 'command'. All the ligand concentrations will remain constant except one of them, which will be assigned a maximum and minimum value by the user. The physiological ligand concentrations will show up on the screen as the reference. The user can choose to change any of them. Then the user will assign the range of one ligand by selecting the ligand and key in the maximum and minimum values. After the calculation, the user can examine the hemoglobin binding curves by selecting the curves of interest.

5. Sample calculations

This section provides several examples for calculating steady state solution, varying enzyme kinetic parameters, integrating the equations, plotting the integration results, and examining enzyme activities as function of some parameters. The users can follow the format of the data file provided here, but it is not necessary to use exactly the same numerical values in these examples. All the data files must be in the same folder as the simulator.

5.1. Steady state solution

After the program is open, the screen will display the main menu and the red blood cell metabolic pathways. The user can examine different parts of the pathways by clicking the scroll bar on the side of the window:



To calculate steady state solution, one must prepare data files 'ss.dat', which contains the initial guess for the steady state solution. The user can create the file before running the program or while running the program using the multifinder. When using the multifinder, 0.5–1.0 MB of RAM memory must be available to the simulator. The format of file 'ss.dat' is specified in the last section of this paper, and the following is an example:

```
33
   .0783238742586310 G6P
   .0214515424943221 F6P
   .0110302900135918 FDP
   .0059496964255317 DHAP
   .0001916107266366 13DPG
   .0957289306390129 PG3
   .0140049424819202 PG2
   .0238447437954066 PEP
   .0603541704974423 PYR
  1.3991010827880620 LAC
  4.1222154428310130 23DPG
   .0299475245109086 NADH
   .0009572218971675 A
   .1501073286614772 AMP
   .4063990004142602 ADP
  1.8845034363260600 ATP
   .0000115981447486 GL6P
   .3180532944225540 G06P
   .0000960091929303 NADP
  3.3197885261668740 GSH
   .0128078773366617 RU5P
   .0328272857613718 R5P
   .0384030233978555 X5P
   .2229783139471450 S7P
   .0005173914906233 E4P
   .0115810568214895 PRPP
   .0180291502069131 IMP
   .0000141149174815 INO
   .0000571424463841 HX
   .0030220580129635 R1P
149.6365524269620000 KI
 12.0280444984959300 NAI
  1.0E-8
  1000
```

Data file 'initial.dat', the content of which is specified in the last section, is also required for the calculation of steady state solution:

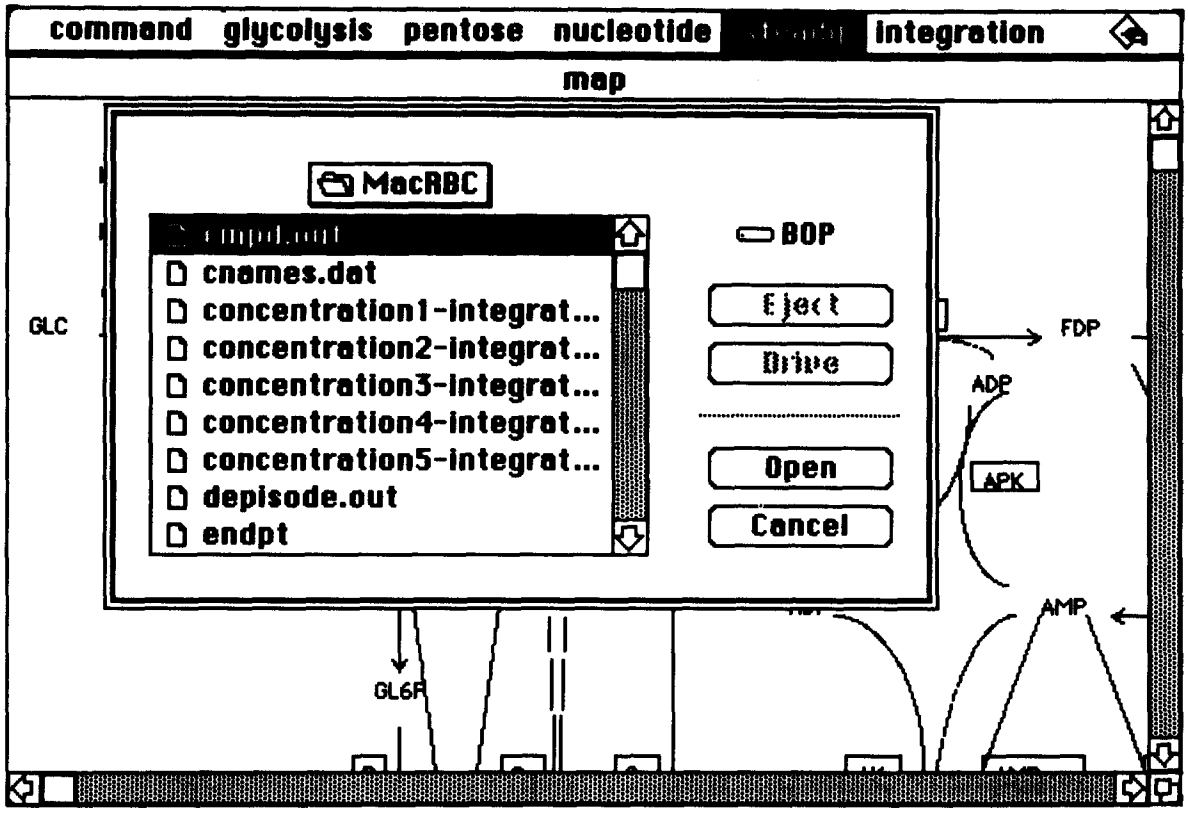
```
300.0 OSM
5.000 GLC
0.013 ADE
1.200 CO2
3.320 GSSGt
1.200 LACext
2.750 Mgt
0.089 NADHt
0.066 NADPHt
1.000 Pi
1.000 PPi
0.060 PYRext
7.300 HB
7.400 pH
```

A file containing the names of metabolites, 'cnames.dat', is also needed.

```
1  'G6P '
2  'F5P '
3  'FDP '
4  'DHAP '
5  'GAP '
6  '13DPG '
7  'PG3 '
8  'PG2 '
9  'PEP '
10  'PYR '
11  'LAC '
12  '23DPG '
13  'NADH '
14  'A '
15  'AMP '
16  'ADP '
17  'ATP '
18  'GL6P '
19  'G06P '
20  'NADP '
21  'GSH '
22  'RU5P '
23  'R5P '
24  'X5P '
25  'S7P '
26  'E4P '
27  'PRPP '
28  'IMP '
29  'INO '
30  'HX '
31  'R1P '
32  'KI '
33  'NAI '
```

To read the initial guess for the steady state solutions, choose item 'read guess values' under menu title 'steady' and open the data file 'ss.dat'.

The ideal initial guess will be the steady state solution for a set of conditions and parameters similar to the current ones. The simulator applies Newton's method to search for the solution; therefore, a bad initial guess may not converge to the true solution. Finally, choose item 'steady state' to calculate the solutions, which will be writen into the output file 'steady-ss.dat'.



5.2. Vary enzyme kinetic parameter

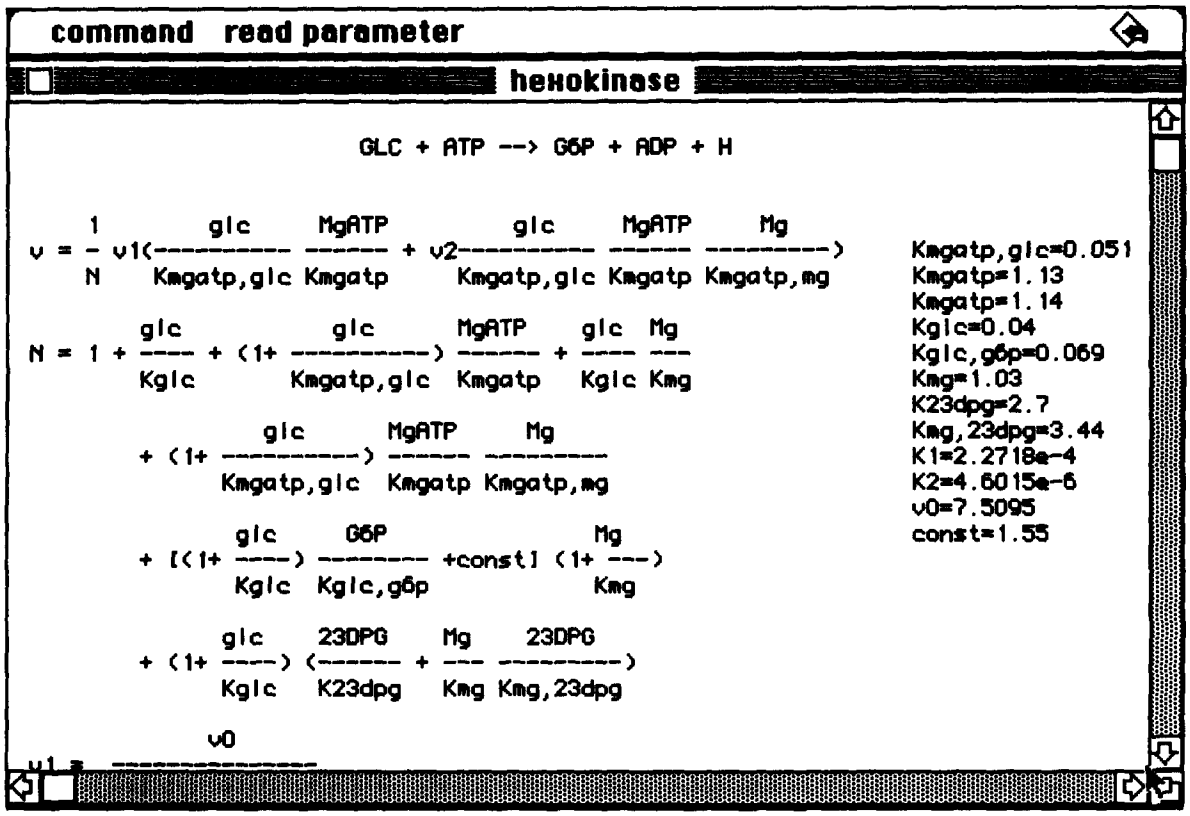
To vary enzyme kinetic parameters, one needs a data file that contains a new set of kinetic parameters for the enzyme of interest. The data file can be prepared using multifinder while running the simulator. In the following example, the parameters Vmax for hexokinase is changed from 7.5095 to 8.0 and saved in file 'hk.dat':

```
0.051
1.13
1.14
0.04
0.069
1.03
2.7
3.44
2.2718e-4
4.6015e-6
8.0
1.55
```

To read the new parameters, click the box for hexokinase in the metabolic pathway and the following screen will appear. On the left of the screen is the reaction, and on the right list the kinetic parameters. The new kinetic parameters in the data file must be in the same order as those shown on the screen.

To change the parameters, choose the item 'read file' under menu title 'read parameter' and open file 'hk.dat'. Follow the procedure for steady state calculation and use 'ss.dat' as the initial guess. The steady state solutions for the new set of kinetic parameters will be saved in file 'steady-ss.dat' and 'hb.output':

```
steady-ss.dat:
    .1071688406618640 G6P
    .0180563312421778 F6P
    .0105257324589483 FDP
    .1279397424648304 DHAP
    .0057975508688860 GAP
    .0001862371033082 13DPG
    .0972614502612578 PG3
    .0142300487685762 PG2
    .0242290453796189 PEP
    .0602856809418147 PYR
```




```
1.3984278719500000 LAC                    .0355244473467394 R5P
4.0026708644642500 23DPG                  .0415515160854491 X5P
 .0299296384356291 NADH                   .2685334045841262 S7P
 .0000616672610864 A                      .0004320588434579 E4P
 .1602156496303170 AMP                    .0130830844878574 PRPP
 .4173848958312820 ADP                    .0190570705186417 IMP
1.8698469281846000 ATP                    .0000153594259180 INO
 .0000115993616484 GL6P                   .0000615014917143 HX
 .4061808271702221 GO6P                   .0032671061318110 R1P
 .0000860942036754 NADP                 149.3609393189443000 KI
3.3197886122968200 GSH                   12.0923518737275499 NAI
 .0138574472908608 RU5P
```

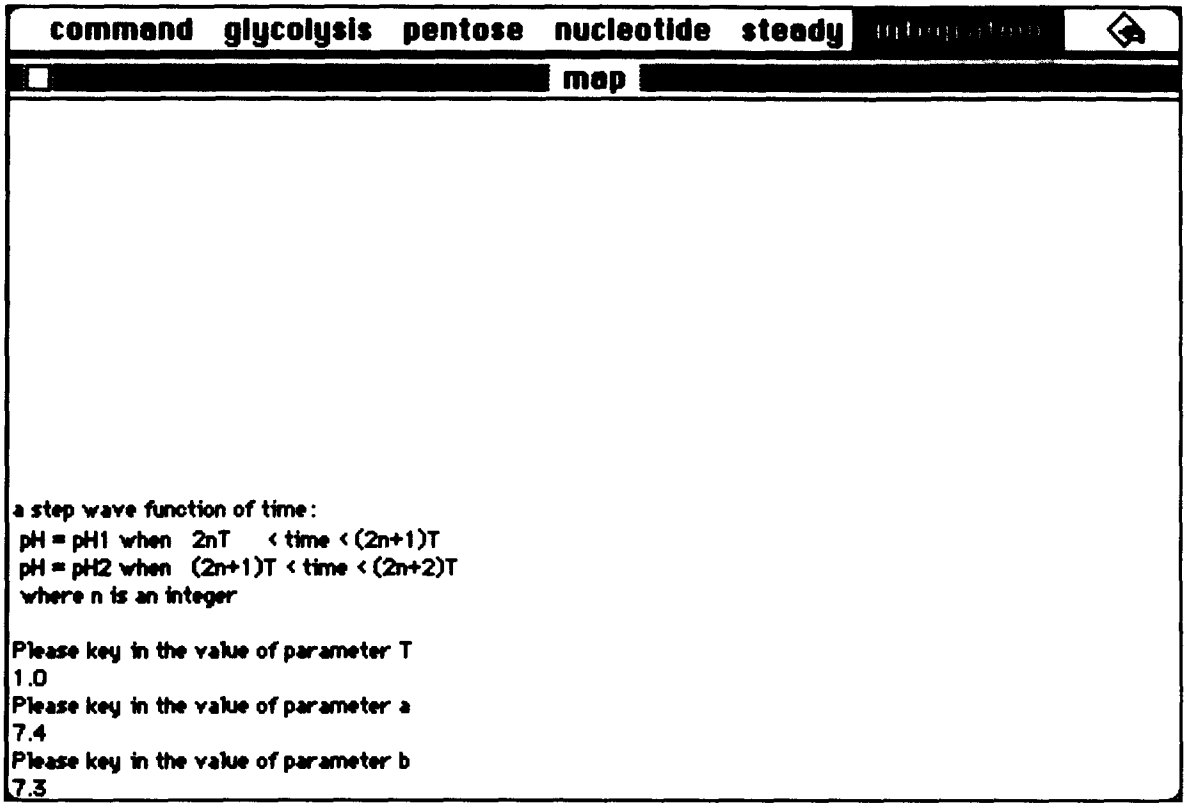
```
hb.output:
   temp     [o2]      [co2]      [232bpgtot] [h]      [c1]
  3.1000E+02  1.000E-03  1.2000E-03  4.1222E-03  6.4885E-08  7.5490E-02
           total fixed charge per hb molecule
 -1.4909E+00
 yhy=78.24 yo2=4.00 yco2= .47 ybpg5= .43 ybpg4=0.00 ybpg3= .40 ycl= .37
```

5.3. Integration

To integrate and obtain the dynamic solutions, one must provide the initial conditions for the integration, for example 'integrate.dat':

```
33
   .0783238742586310 G6P
   .0214515424943221 F6P
   .0110302900135918 FDP
   .1312854805868560 DHAP
   .0059496964255317 GAP
   .0001916107266366 13DPG
   .0957289306380129 PG3
   .0140048424819202 PG2
   .0238447437954066 PEP
   .0603651704974423 PYR
  1.3991010827880620 LAC
  4.1222154428310130 23DPG
   .0299475245109086 NADH
   .0000572218971675 A
   .1501073286614772 AMP
   .4063990004142602 ADP
  1.8845034363260600 ATP
   .0000115981447486 GL6P
   .3180532944225540 G06P
   .0000960091929303 NADP
  3.3197885261668740 GSH
   .0128078773366617 RU5P
   .0328272857613718 R5P
   .0384030233978555 X5P
   .2229783139471450 S7P
   .0006173914906233 E4P
   .0115801568214895 PRPP
   .0180291502069131 IMP
   .0000141149174815 INO
   .0000571424463841 HX
   .0030220580129635 R1P
149.6365524269520000 KI
 12.0280444984959300 NAI
0.0
2.0
100
1d-2
```

The user can also assign perturbation on pH, osmolarity, and other conditions to the red cell by changing the data file 'initial.dat'. After 'integrate.dat' is edited, choose item 'read initial value' under mean title 'integrate' to read the initial conditions. Then choose one of the time-dependent functions of external parameter under menu title 'integrate', for example, 'pH wave', and key in the period and altitude of the wave function as follows:

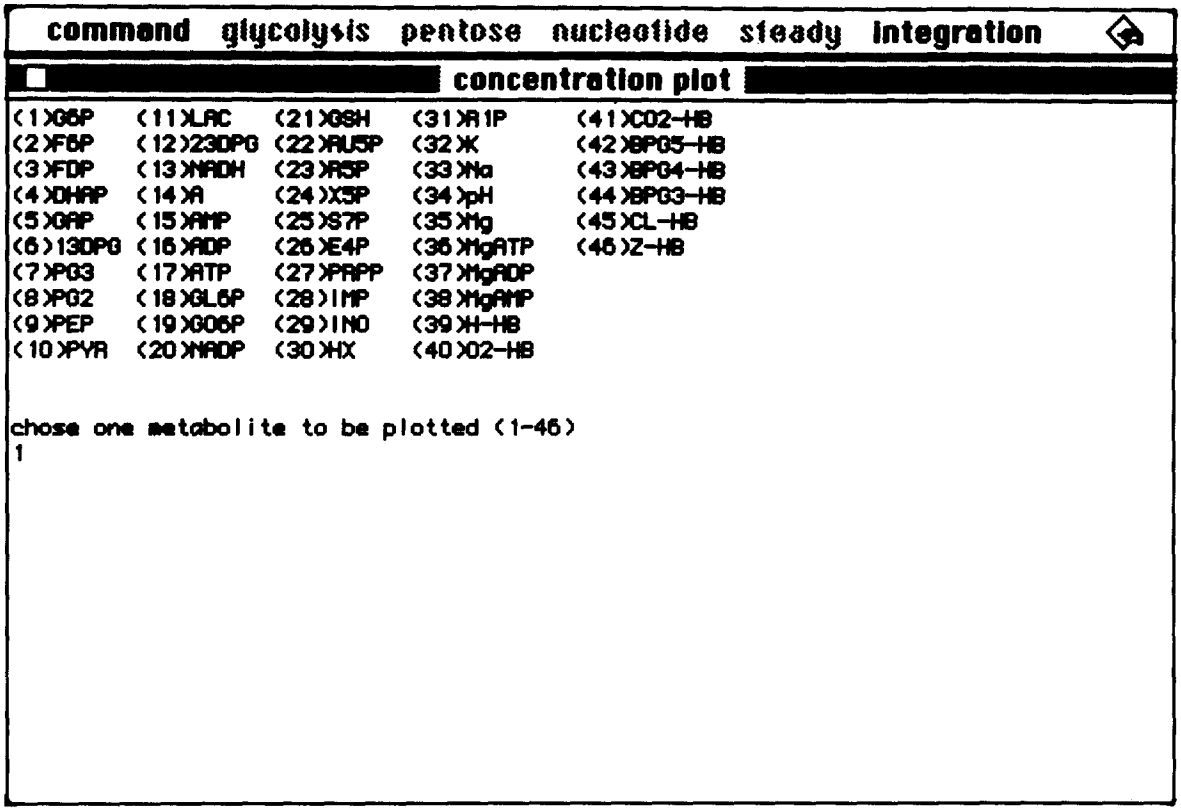


The CPU time for integration depends on the time length and the accuracy specified by the user and how far the initial point is away from the steady state condition. The first point will take the longest CPU time to integrate, and the integration of the latter points will be significantly faster. However, the calculation around the discontinuous points of the wave function will also be slow. The integration results will be saved into several files: the metabolite concentrations in 'concentration1-integrate.dat', 'concentration2-integrate.dat' and so on, an enzyme fluxes in 'flux1-integrate.dat', 'flux2-integrate.dat', and so on:

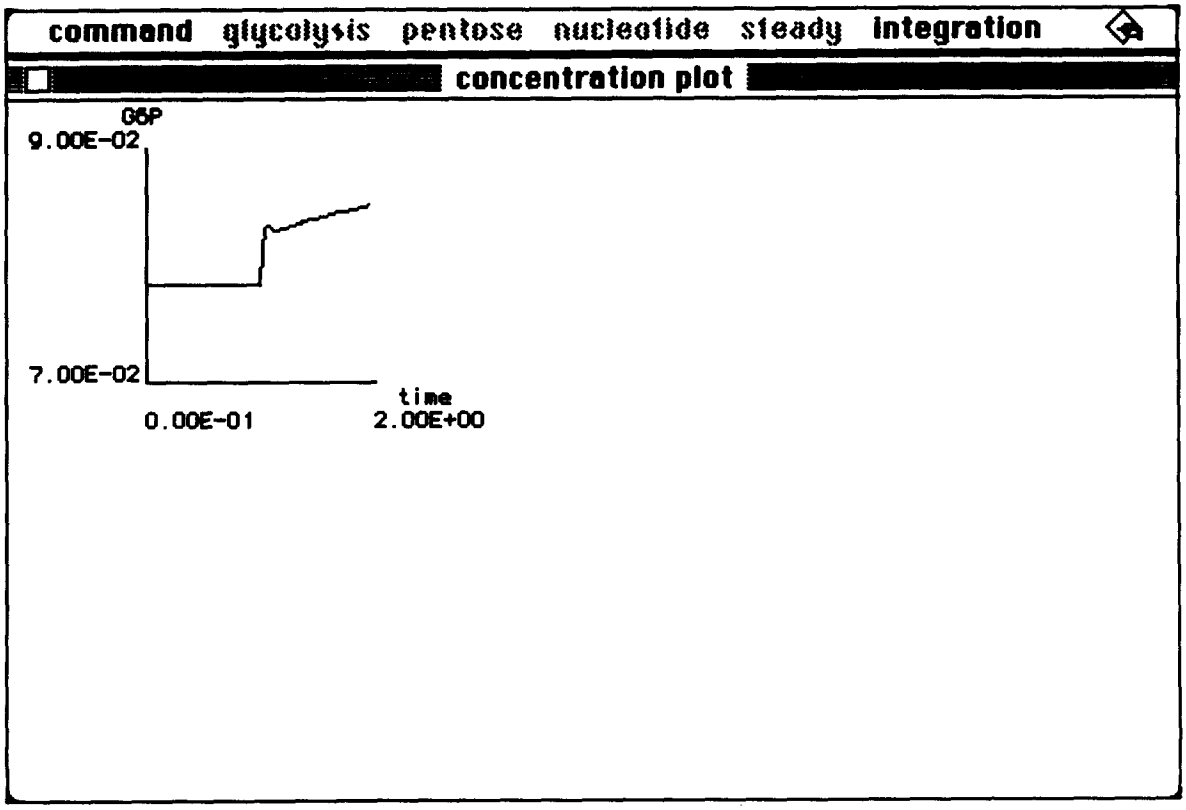
```
concentration1-integrate.dat
   time       G6P          F6P          FDP         DHAP          GAP          PG13         PG3
PG2...
0 0.000E-01 7.8324E-02 2.1452E-02 1.1030E-02 1.3129E-01 5.9497E-03 1.9161E-04 9.5729E-02
1.4005E-02
1 2.000E-02 7.8324E-02 2.1452E-02 1.1030E-02 1.3129E-01 5.9497E-03 1.9161E-04 9.5729E-02
1.4005E-02
2 4.000E-02 7.8324E-02 2.1452E-02 1.1030E-02 1.3129E-01 5.9497E-03 1.9161E-04 9.5729E-02
1.4005E-02
3 6.000E-02 7.8324E-02 2.1452E-02 1.1030E-02 1.3129E-01 5.9497E-03 1.9161E-04 9.5729E-02
1.4005E-02
4 8.000E-02 7.8324E-02 2.1452E-02 1.1030E-02 1.3129E-01 5.9497E-03 1.9161E-04 9.5729E-02
1.4005E-02
5 1.000E-01 7.8324E-02 2.1452E-02 1.1030E-02 1.3129E-01 5.9497E-03 1.9161E-04 9.5729E-02
1.4005E-02
...
...
```

```
flux1-integrate.dat
                                                                    time       HK           PG1
PFK          ALD          TPI          GAPDH          PGK          DPGM...
0 0.000E-01 1.2853E+00 1.5702E+02 1.2125E+00 1.2125E+00 1.2125E+00 2.4978E+00 2.0018E+00
4.9608E-01
1 0.000E-02 1.2853E+00 1.5702E+02 1.2125E+00 1.2125E+00 1.2125E+00 2.4978E+00 2.0018E+00
4.9608E-01
2 0.000E-02 1.2853E+00 1.5702E+02 1.2125E+00 1.2125E+00 1.2125E+00 2.4978E+00 2.0018E+00
4.9608E-01
3 0.000E-02 1.2853E+00 1.5702E+02 1.2125E+00 1.2125E+00 1.2125E+00 2.4978E+00 2.0018E+00
4.9608E-01
4 0.000E-02 1.2853E+00 1.5702E+02 1.2125E+00 1.2125E+00 1.2125E+00 2.4978E+00 2.0018E+00
4.9608E-01
5 0.000E-01 1.2853E+00 1.5702E+02 1.2125E+00 1.2125E+00 1.2125E+00 2.4978E+00 2.0018E+00
4.9608E-01
```

To plot the integration results as functions of time, choose the item 'plot concentration' or 'plot flux' under menu title 'integration', and select one of the metabolites or fluxes of interest.



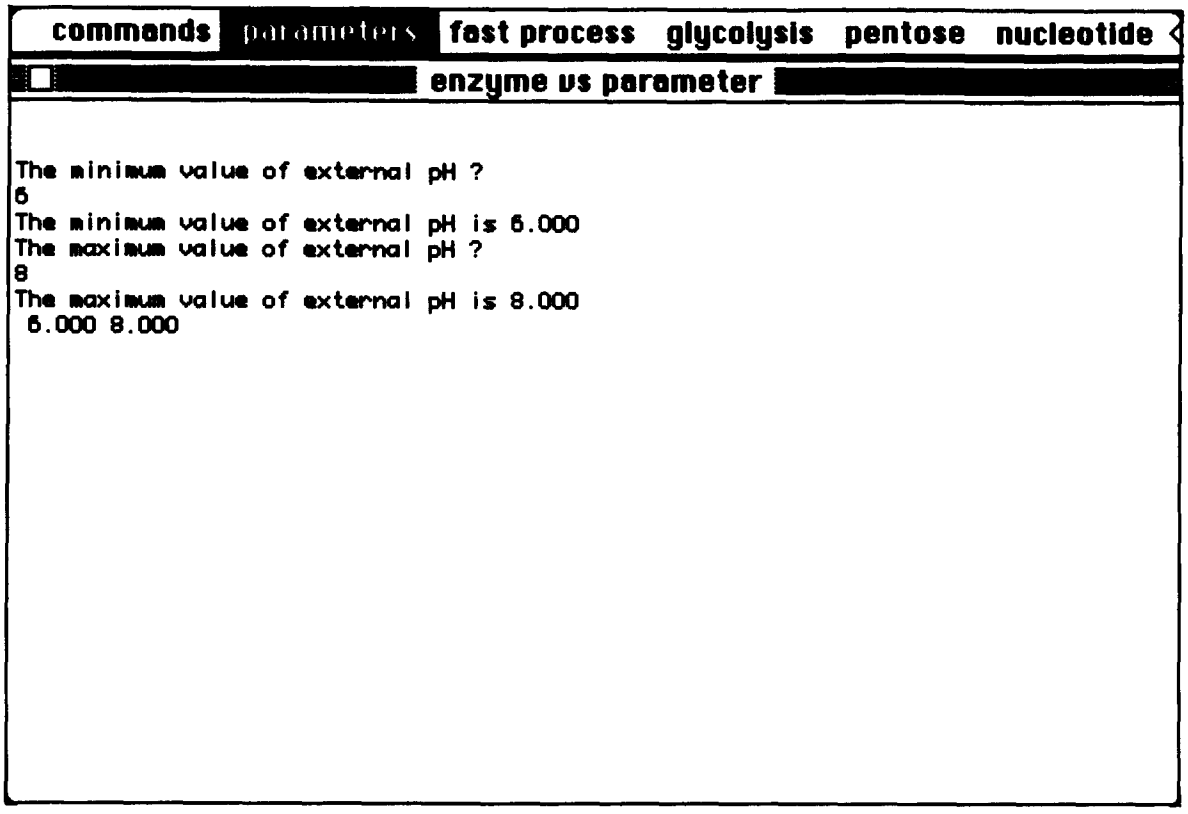
For example, choose 1 to plot G6P concentration as a function of time. The plot will be shown on the screen as follows.



More sophisticated plots may be obtained by reading and reformating the output files from other graphic software packages.

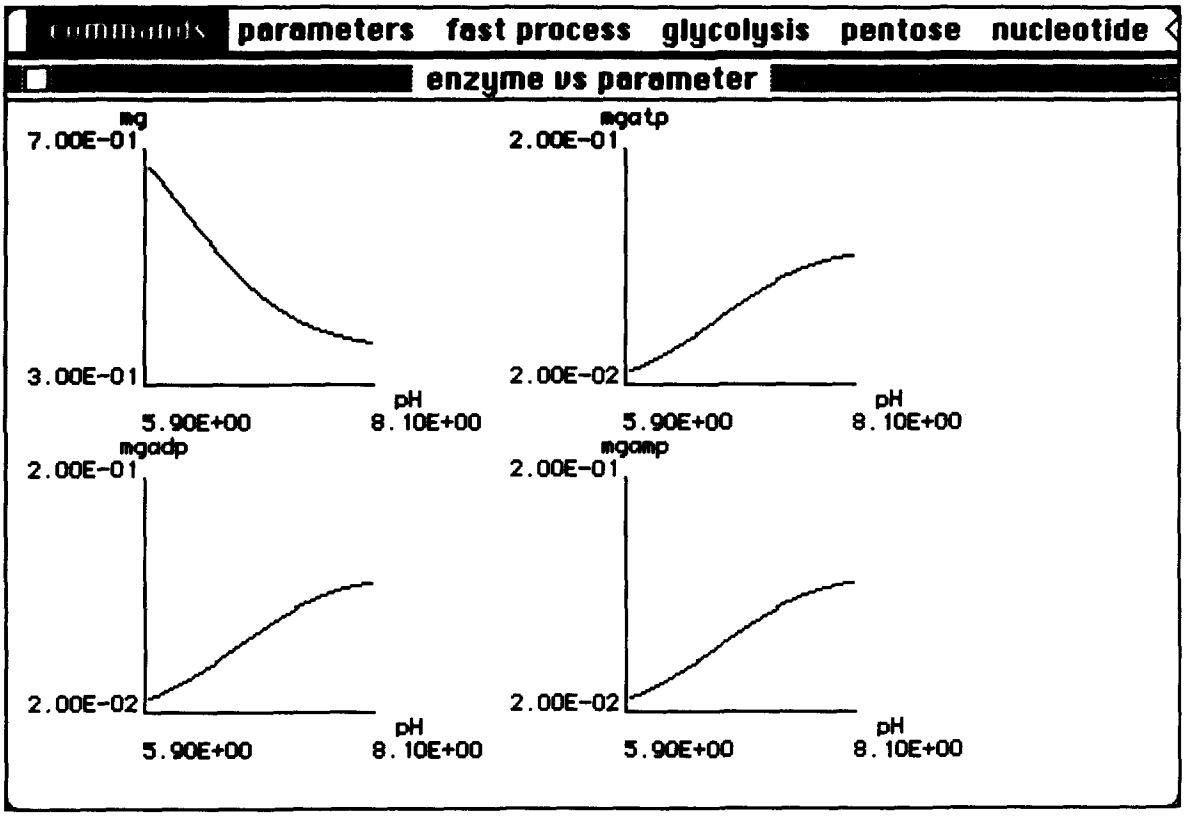
5.4. Enzyme activity vs parameter

To calculate enzyme activity as a function of a parameter, simply choose item 'enzyme vs parameter' under menu title 'command'. However, this calculation can be performed only after a steady state solution is obtained, since the calculation is based on the reference metabolite concentrations given in 'steady-ss.dat' or whatever file containing the steady state solutions. Choose the parameter under 'parameter' and give the range of the parameter. For example, pH:



Then select one of the processes or enzyme fluxes under 'fast process', 'glycolysis', 'pentose', or 'nucleotide'. To see the correlation, choose 'calculate response' under menu title 'commands' and the

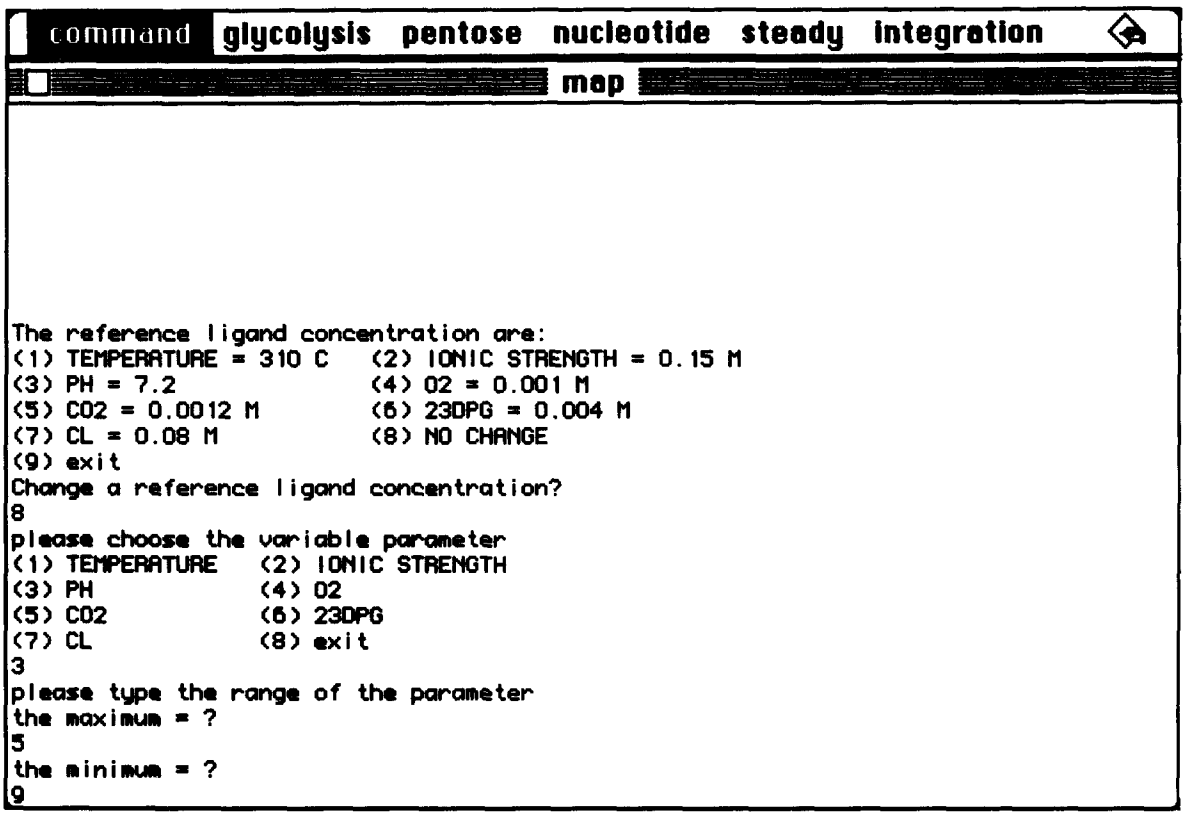
results will be plotted on the screen. The following are Mg complexes as functions of pH varying from 6.0 to 8.0:



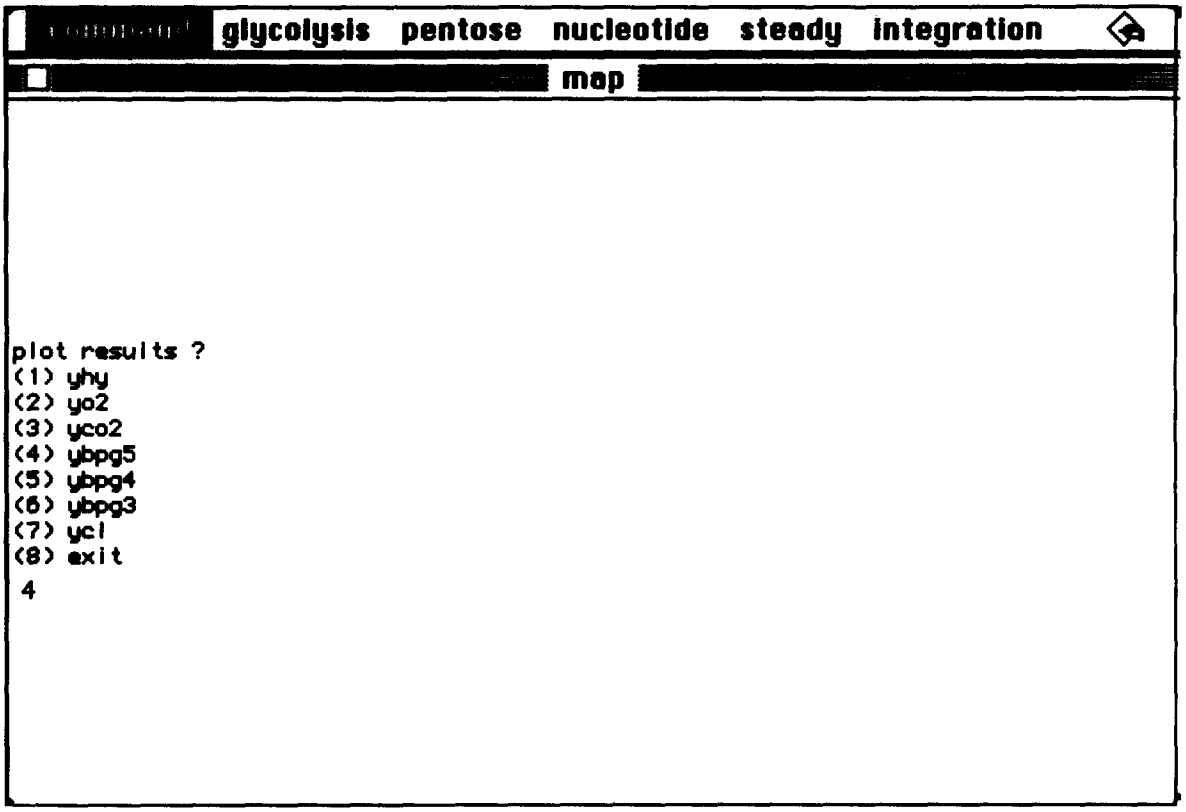
5.5. Hemoglobin binding curve

To calculate the hemoglobin binding curves, choose 'hemoglobin binding' under 'command', and a list of reference ligand concentrations will appear. Change the reference ligand concentrations by typing the corresponding number and keying in the new concentration, or type 8 to continue to next step.

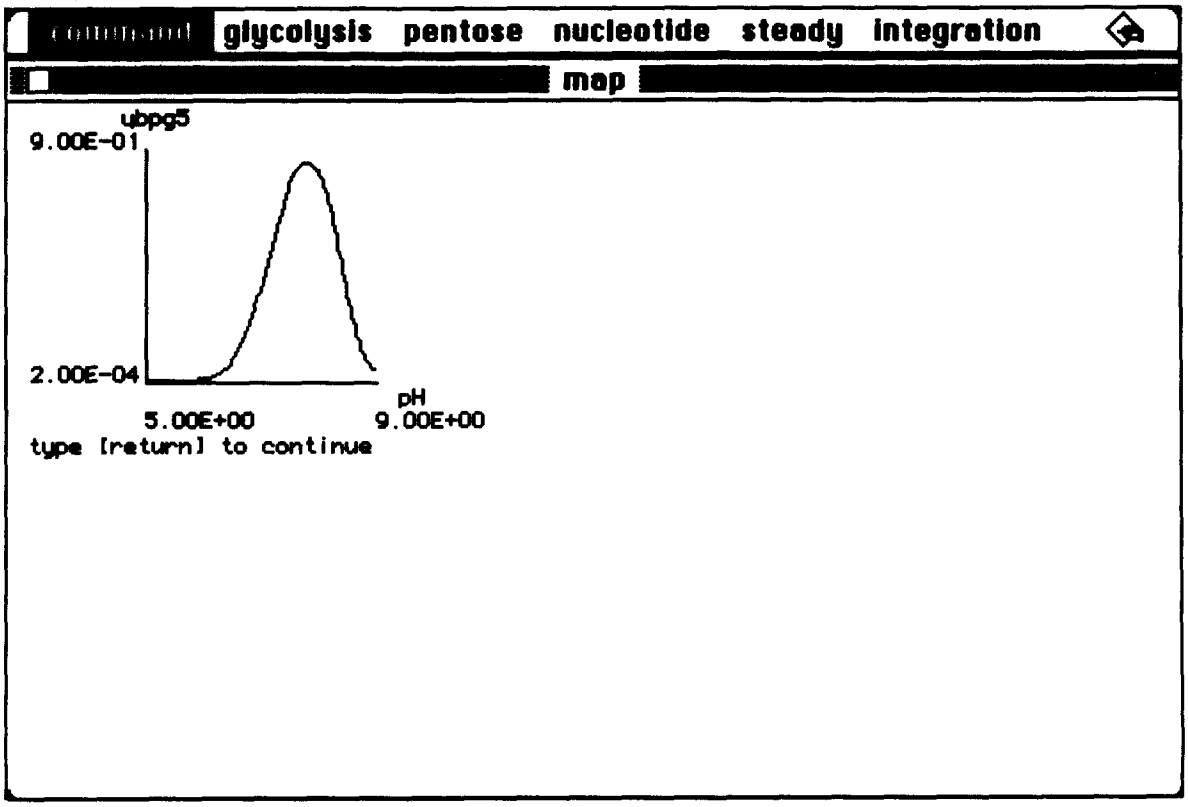
Then select the variable ligand concentration and give the maximum and minimum values. In the above example, pH is varied from 5 to 9. After a few seconds of calculation, the following will show up on the screen:



Type 4 to plot $23DPG^{5-}$ binding coefficience as a function of pH



To plot another ligand binding curve, type 'return' and choose a ligand.



6. Simulation results

This section presents simulation results calculated from the model. These calculations also illustrate the utility of the red cell metabolic model.

6.1. Steady state metabolite concentrations

One of the primary capabilities of this program is to predict the steady state metabolic concentrations under a given condition. This calculation can be applied to study the red cell metabolism under (1) enzyme deficiency: where any kinetic parameter of the 43 red cell enzymes can be varied in the model, (2) altered extracellular conditions: such as pH, osmolarity, glucose, and lactate, and (3) abnormal intracellular hemoglobin, magnesium, total GSH, NADH, and NADPH concentrations. The physiological steady state metabolic concentrations are calculated and compared with the literature data (Table 11). The calculated numbers agree well with the experimental data. It is worthwhile to note that the intracellular concentration data for human red cell may vary from one subject to another due to

TABLE 11

Steady state concentration of red cell intermediates

Metabolite	Model (mM)	Literature (mM)	Reference
G6P	0.040	0.038 ± 0.012	[20,21]
F6P	0.019	0.016 ± 0.003	[20,21]
FDP	0.011	0.0076 ± 0.004	[20,21]
DHAP	0.13	0.14 ± 0.08	[20,21,22]
GA3P	0.0059	0.0067 ± 0.001	[20,21]
13DPG	0.00019	0.0004	[20,21]
3PG	0.095	0.045	[20,21]
2PG	0.014	0.014 ± 0.005	[20,21]
PEP	0.024	0.017 ± 0.002	[20,21]
PYR	0.059	0.077 ± 0.05	[20,21,23,22]
LAC	1.36	1.10 ± 0.50	[20,21]
2,3DPG	4.02	4.5 ± 0.5	[24,21,22]
NADH	0.0295	†	[23,25,26]
A	0.000057	0.0012 ± 0.0003	[25]
AMP	0.148	0.080 ± 0.009	[20,21]
ADP	0.39	0.27 ± 0.12	[20,21]
ATP	1.72	1.54 ± 0.25	[20,21]
GL6P	0.000011	†	†
GO6P	0.186	†	†
NADPH	0.0648	0.0658	[26]
GSH	3.32	3.21 ± 1.5	[20]
RU5P	0.0129	†	†
R5P	0.033	†	†
X5P	0.039	†	†
S7P	0.23	†	†
E4P	0.00047	†	†
PRPP	0.012	0.005 ± 0.001	[27,28]
IMP	0.018	0.010	[27,28]
INO	0.000014	0.001	[27,28]
HX	0.000057	0.002	[27,28]
R1P	0.003	0.06	[27,28]
K	146.0	135.0 ± 10.0	[29,30]
Na	12.7	10.0 ± 6.0	[29,30]

† Exact experimental value is difficult to determine and is not readily available.

various genetic or pathological reasons, and the data in Table 11 are for a typical human [18].

6.2. *Hemoglobin binding affinity with ligands*

The major function of red blood cell is to transport oxygen from the lungs to tissues, and deliver carbon dioxide in the reverse direction. Therefore, it is important to be able to predict the hemoglobin binding affinity with its ligands under various conditions. The physiological ligands of hemoglobin molecule includes O_2, CO_2, Cl^-, 23 DPG, and H^+. The interactions between all five ligands with the hemoglobin molecule are accounted for in the metabolic model. The hemoglobin oxygen binding affinity (Y_{O_2}) under different 23DPG concentrations calculated from the red cell model is shown in Fig. 4. The calculations compare well with the experimental data as discussed in [17]. The results indicate that the hemoglobin molecule becomes more rigid and has less affinity with O_2 when 23DPG concentration increases.

6.3. *Metabolic response to extracellular perturbation*

The extracellular conditions of red cell may not be constant under experimental or physiological conditions. The red cell metabolic model is capable of predicting the metabolic response of red cell to a perturbation of extracellular glucose, pH, osmolarity, lactate, or pyruvate. For example: The normal blood glucose concentration is 5 mM [20] and rises to 8 mM after a meal. Suppose a subject eats a meal every 8 h, and his/her blood glucose level remains at 8 mM for 4 h after a meal and then drops to 5 mM until the next meal. As a result, the blood glucose concentration follows a periodic perturbation shown in Fig. 5. The response of red cell intracellular G6P and ATP to the glucose perturbation are calculated from the model and shown in Fig. 6. The results indicate that G6P responds with a significant variation since it is a direct metabolite produced from glucose by enzyme hexokinase. However, the red cell is able to maintain a stable energy (ATP) level regardless of the glucose perturbation.

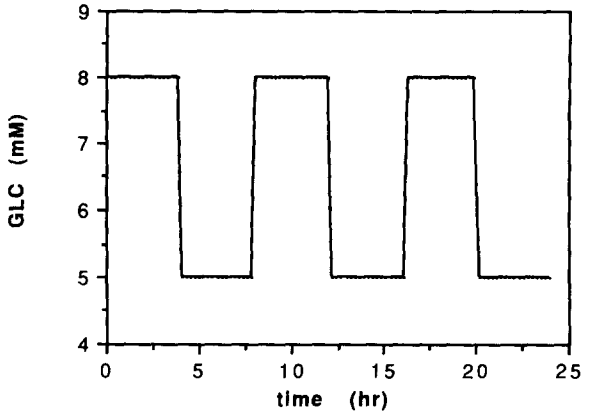


Fig. 5. Suppose a subject eats a meal every 8 h, and his/her blood glucose level remains at 8 mM for 4 h after a meal and then drops to 5 mM until the next meal. As a result, the blood glucose concentration follows a periodic perturbation.

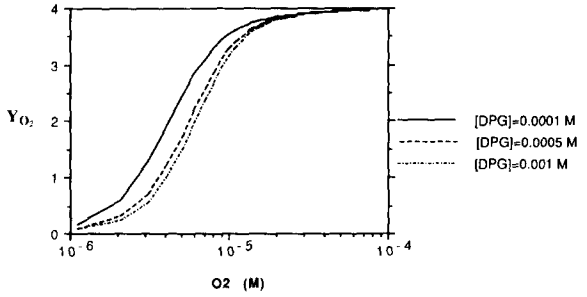


Fig. 4. The hemoglobin binding affinity with oxygen under different 23DPG concentrations.

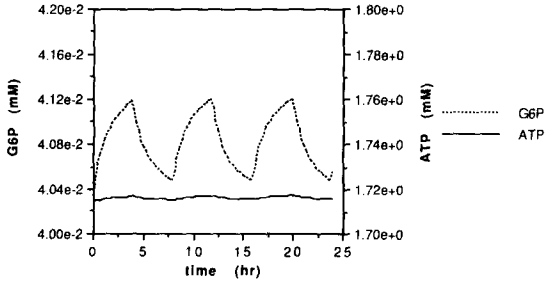


Fig. 6. The response of G6P and ATP to a periodic perturbation of extracellular glucose level.

7. Hardware and software specification

This software package is written for a Macintosh computer with 68020 processor and 68881 coprocessor (such as Mac II and Mac IIci). The memory requirement for this program is 1 MB. Data files can be edited by a text file editor such as Edit™.

Acknowledgement

This work has been supported by National Institute of Health (grant no. DK 39256).

References

[1] T.A. Rapoport, R. Heinrich, G. Jacobasch and S. Rapoport, Eur. J. Biochem. 42 (1974) 107–120.

[2] T.A. Rapoport and R. Heinrich, Mathematical analysis of multienzyme systems; i. modeling of glycolysis of human erythrocytes, Biosystems 7 (1975) 120–129.

[3] T.A. Rapoport, R. Heinrich and S.M. Rapoport, The regulatory principles of glycolysis in erythrocytes in vivo and in vitro. Biochem. J. 154 (1976) 449–469.

[4] R. Heinrich, S.M. Rapoport and T.A. Rapoport, Metabolic regulations and mathematical models, Prog. Biophys. Mol. Biol. 32 (1977) 1–82.

[5] F.I. Ataullakhanov, V.M. Vitvitsky, A.M. Zhabotinsky, A.V. Pichugin and O.V. Platonova. The regulation of glycolysis in human erythrocytes, Eur. J. Biochem. 115 (1981) 359–365.

[6] M. Schauer, R. Heinrich and S.M. Rapoport, Mathematische modellierung der glykolyse und des adeninnukleotidstoffwechsels menschlicher erythrozyten. 1. reactionskinetische anatze, analyse des in vivo-zustandes and bestimmung der anfangshedingungen fur die in vitro-experimente, Acta Biol. Med. Germ. 40 (1981) 1659–1682.

[7] M. Brumen and R. Heinrich, A metabolic osmotic model of human erythrocytes, Biosystems 17 (1984) 155–169.

[8] H.G. Holzhutter, G. Jacobasch and A. Bisdorff, Mathematical modeling of metabolic pathways affected by an enzyme deficiency, Eur. J. Biochem. 149 (1985) 101–111.

[9] A. Werner and R. Heinrich, A kinetic model for the interaction of energy metabolism and osmotic states of human erythrocytes, analysis of the stationary in vivo state and of time dependent variations under blood preservation conditions, Biomed. Biochim. Acta 44 (1985) 185–212.

[10] A. Joshi and B.O. Palsson, Metabolic dynamics in the human red cell, part I—a comprehensive model, part II —interactions with the environment, J. Theor. Biol. 141 (1989) 515–545.

[11] A. Joshi and B.O. Palsson, Metabolic dynamics in the human red cell, part III—metabolic reaction rates, part IV—data prediction and some model computations, J. Theor. Biol. 142 (1990) 41–85.

[12] I-Der Lee and B.O. Palsson, A comprehensive model of human erythrocyte metabolism: Extension to include pH effects. Biomed. Biochim. Acta, 8/9 (1990) 771–789.

[13] J.C. Freedman and J.F. Hoffman, Ionic and osmotic equilibria of human red blood cells treated with nystatin, J. Gen. Physiol. 74 (1979) 157–185.

[14] H. Passow, Ion and water permeability of the red blood cell, In: The Red Blood Cell, eds. C. Bishop and D.M. Surgenor (1964).

[15] J.R. Sachs, P.A. Knauf, and P.B. Dunham, Transport through red cell membranes. The Red Blood Cell, ed. D.M. Surgenor, PP. 613–703 (Academic Press, New York San Francisco and London, 1975).

[16] J.W.R. Lawson and R.L. Veech, Effect of pH and free Mg^{++} on the K_{eq} of the creatine kinase and other phosphate hydrolysis and phosphate transfer fluxes, J. Biol. Chem. 254 (1979) 6528–6537.

[17] T. Yoshida and M. Dembo, A thermodynamic model of hemoglobin suitable for physiogical applications (Modeling Methodology Forum, American Physiological Society, pp. C563–C577, 1990).

[18] D.O. Cooney, Biomedical Engineering Principles (Marcel Dekker, Inc., New York and Basel, 1976).

[19] L.M. Mcintyre, D.R. Thorburn, W.A. Bubb and P.W. Kuchel, Comparison of computer simulations of f-type and l-type non-oxidative hexose monophosphate shunts with ^{31}P-nmr experimental data from human erythrocytes, Eur. J. Biochem. 180 (1989) 399–420.

[20] E. Beutler, Red Cell Metabolism (Grune & Stratton, New York, second edition, 1975).

[21] G.S. Jacobasch, S. Minakami and S.M. Rapoport, Cellular and Molecular Biology of Erythrocytes, pp. 55–92 (University of Tokyo Press, Tokyo, 1974).

[22] I. Rapoport, S.M. Rapoport, D. Maretzki and R. Elsner. The breakdown of adenine nucleotides in glucose depleted human red cells, Acta Biol. Med. Germ. 38 (1979) 1419–1429.

[23] W.E. Marshall and U.A. Omachi, Biochim. Biophys. Acta, 354 (1974) 1–10.

[24] G. Gerber, H. Berger, G.R. Janig, K. Ruckpaul and S.M. Rapoport, Vii. internationale symposium uber struktur und funktion der erythrozyten, S.M. Rapoport and F. Jung, eds, pp. 272–282 (Akademie-Verlag, Berlin, 1975).

[25] G.C. Mills, F.C. Schmaltieg, K.B. Trimmer, A.S. Goldman and R.M. Goldblum, Purine metabolism in deaminase deficiency, Proc. Natl. Acad. Sci. USA 73 (1976) 2867–2871.

[26] A. Omachi, C.B. Sott and H. Hegarty, Biochim. Biophys. Acta 184 (1969) 139–147.

[27] I.H. Fox and W.N. Kelley, Phosphoribosylpyrophosphate in man: biochemical and clinical significance, Ann. Inter. Med. 75 (1971) 424–433.

[28] J.F. Henderson and A.R.P. Paterson, Nucleotide Metabolism: An Introduction (Academic Press, New York and London, 1973).

[29] J. Funder and J.O. Weith, Acta Physiol. Scand. 68 (1966) 234–235.

[30] S. Hellerstein, W. Spees and L.O. Surapathana, J. Lab. Clin. Med., 76 (1970) 10–24.