A mathematical description of PO_4-transport in the red blood cell

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KURZFASSUNG: **Eine mathematische Beschreibung des PO_4-Transports in der roten Blutzelle.** Für den PO_4-Austausch in den roten Blutzellen gelten hinsichtlich des Gesamtphosphors folgende Beziehungen, und zwar sowohl im Normalfall als auch bei Sichelzellenanämie:

$$x_0 = (x_{0,0} - x_{0,\infty})e^{-\alpha t} + x_0$$

$$x_1 = x_{0,\infty} - \frac{k'}{k}(x_{0,0} - x_{0,\infty})e^{-\alpha t}$$

Hierin bedeutet x spezifische Aktivität, 0 bezieht sich auf das Plasma und 1 auf das Innere der roten Blutzelle; $x_{0,0}$ ist der Anfangswert und $x_{0,\infty}$ der Wert nach einer sehr langen Zeit. Diese Ausdrücke repräsentieren die Lösungen von gekoppelten linearen Differentialgleichungen. Durch Einsetzen der gemessenen Werte von Gesamtphosphat und anorganischem Phosphat beziehungsweise der Verhältnisse der spezifischen Aktivität in der Normal- und Sichelzelle können die Transportgeschwindigkeiten berechnet werden; auch lassen sich mit Hilfe dieser Formel die brauchbaren Parameter gewinnen, an Hand derer die Unterschiede zwischen Normal- und Sichelzelle diskutiert werden können. Diese Unterschiede werden bei Anwendung von Inhibitoren besonders deutlich, wobei ein Hemmquotient

$$\frac{(x_1/x_0)_{\text{Kontrolle}}}{(x_1/x_0)_{\text{Agens}}}$$

definiert wird. Die Daten reichen zur Modellvorstellung eines 3-Kompartimentsystems nicht aus.

INTRODUCTION

In studying the differences between normal and sickle cell anemic red blood cells (r.b.c.), we are investigating variations in the transport of various substances and how these transports are influenced by various chemical agents and physical factors. The discussion of our data requires some model of transport from which parameters can be derived. We illustrate our procedures through results obtained by studying phosphorus transport with ^{32}P labeled NaH_2PO_4.

METHODS AND RESULTS

The experimental part consisted of incubating red blood cells with $NaH_2{}^{32}PO_4$ in a constant temperature Warburg apparatus. The details of the experiment are

essentially the same as from experiments reported earlier (LABAT et al. 1958). The results needed for this paper are summarized in Table 1.

Table 1

Amounts of phosphorus in the blood in mg per 100 ml

Object	Total phosphorus	Inorganic phosphorus
Normal		
Plasma	22.25	2.28
Red blood cells	102.36	3.24
Sickle cell		
Plasma	20.05	2.22
Red blood cells	89.72	10.19

In Table 2 are the data for the transport of phosphorus for normal and sickle cell samples. The primary focus of this paper is to outline how these data are interpreted.

The model

The model most appropriate for the experimental data depicts the red blood cell as a two compartment system, designated simply the outside, region "0" (the plasma), and the inside, region "1". The system of equation postulated is then:

$$\begin{aligned} \frac{d\,(m_0 x_0)}{dt} &= \varrho_{10}\, x_1 - \varrho_{01}\, x_0 \\ \frac{d\,(m_1 x_1)}{dt} &= \varrho_{01}\, x_0 - \varrho_{10}\, x_1 \end{aligned} \tag{1}$$

where m signifies the amount of P in milligrams per 100 milliliters of blood and x denotes the specific activities in counts per minute per milligram of P. The P's are transport coefficients in units of minutes^{-1}. The assumption of a steady state for the transport of P and the requirement that the specific activities will equilibrate after a long time, yield

$$\begin{aligned} \frac{dx_0}{dt} &= k\,(x_1 - x_0) \\ \frac{dx_1}{dt} &= k'\,(x_0 - x_1) \end{aligned} \tag{2}$$

where

$$\begin{aligned} \frac{\varrho_{10}}{m_0} &= \frac{\varrho_{01}}{m_0} = k \\ \frac{\varrho_{01}}{m_1} &= \frac{\varrho_{10}}{m_1} = k' \end{aligned} \tag{3}$$

The set of equations (2) is easily solved by differentiating the first and substituting.

This process leads to a second order differential equation with constant coefficients without a first derivative term. The solution is

$$x_0 = (x_{0,0} - x_{0,\infty})\, e^{-at} + x_{0,\infty} \tag{4}$$

with $a = k + k'$

and $x_{0,0}$ designating the specific activity outside at time, t = 0. For the specific activity within the r. b. c. substitution yields

$$x_1 = x_{0,\infty} - \frac{k'}{k}(x_{0,0} - x_{0,\infty})\, e^{-at} \tag{5}$$

Since at infinite time, $x_{1,\infty} = x_{0,\infty}$ and at t = 0, $x_{1,0} = 0$,

$$(k + k')\, x_{0,\infty} = k'\, x_{0,0} \tag{6}$$

Interpretation of results

The data in Table 2 give the ratio of x_0/x_1 so that if equation (4) is divided by equation (5) we have:

$$\frac{x_0}{x_1} = \frac{(x_{0,0} - x_{0,\infty})\, e^{-at} + x_{0,\infty}}{x_{0,\infty} - \frac{k'}{k}(x_{0,0} - x_{0,\infty})\, e^{-at}} \tag{7}$$

Table 2

Ratios of specific activities for normal and sickle cell blood samples

Object	Initially	4 hours	Infinite time
Normal			
$\frac{x_0}{x_1}$		1.64	1
Sickle cell			
$\frac{x_0}{x_1}$		2.87	1

The ratio after 4 hours is $x_0/x_1 = 1.64$. The amounts $m_0 = 2.28$ mg of P/100 ml in the plasma and $m_1 = 3.24$ mg of P/100 ml in the red blood cell are the averages for the normal samples. Thus the radioactivity in the plasma is normalized to $m_0 x_0 = 2.28 \times 1.64 = 3.75$, and within the red blood cell to $m_1 x_1 = 3.24$, so that initially all of this radioactivity was in the plasma, that is, $m_0 x_{0,0} = 6.99$; from which $x_{0,0} = 3.06$. Finally this total radioactivity is distributed, so as to give the same specific activity within is without, thereupon $x_{0,\infty} = 1.23$, which is the same requirement for the conservation of radioactivity as

$$(m_0 + m_1)\, x_{0,\infty} = m_0\, x_{0,0} \tag{8}$$

so that $x_{0,\infty} = 0.413\, x_{0,0}$. Then, too, equations (6) and (8) require

$$\frac{m_0}{m_0 + m_1} = \frac{k'}{k + k'}, \quad \frac{k'}{k} = \frac{m_0}{m}$$

With these values we turn to equation (7) which on substituting and simplifying reduces to

$$1.64 = \frac{1 + \left[\frac{x_{0,0}}{x_{0,\infty}} - 1\right] e^{-4\alpha}}{1 - \frac{k'}{k}\left[\frac{x_{0,0}}{x_{0,\infty}} - 1\right] e^{-4\alpha}}$$

Furthermore from equation (8)

$$\frac{x_{0,0}}{x_{0,\infty}} - 1 = \frac{m_0 + m_1}{m_0} - 1 = \frac{m_1}{m_0}; \quad \text{also} \quad \frac{k'}{k}\left(\frac{x_{0,0}}{x_{0,\infty}} - 1\right) = \frac{k'}{k} \cdot \frac{m_1}{m_0} = 1$$

and as the final step in the substitutions, $\alpha = k + k' = k'\left(1 + \frac{m_1}{m_0}\right)$ so that

$$1.64 = \frac{1 + 1.42\, e^{-4}}{1 - e^{-4}} \tag{10}$$

With $y = 2.42\ Tk'$ where T = 240 minutes, equation 10 is easily solved by referring to a table of exponentials to give $k' = 2.63 \times 10^{-3}$ minutes^{-1}. With this value of k', we have for the transport of P as inorganic P across the red blood cell membrane,

$$\varrho_{10} = \varrho_{01} = m_1 k' = 8.65 \times 10^{-3} \quad \frac{\text{mgms. of P}}{\text{100 milliliters of blood} \times \text{minute}}$$

Repeating the steps with the data for the sickle cell anemic samples for which m_0 = 2.22 mg/100 ml of blood; m_1 = 10.19 mg/100 ml of blood. At the end of four hours, the ratio is $\frac{x_0}{x_1} = 2.87$. Proceeding exactly as before we have corresponding to equation (10), $2.87 = \frac{1 + 4.60\, e^{-4}}{1 - e^{-4}}$ with $y = 5.60\ k'T$ and T = 240 minutes. The value for k' is found to be $k' = 1.03 \times 10^{-3}$ minute^{-1} and the transport of P as inorganic P in each 100 ml of blood is

$$\varrho_{01} = \varrho_{10} = k' m_1 = 10.5 \times 10^{-3} \quad \frac{\text{milligrams}}{\text{minute}}$$

Thus transport of P in the normal red blood cell (8.05 μg/min) does not differ markedly from the sickle cell red blood cell (10.5 μg/min).

The analog computer solution

The physical processes represented in equations (1) may be simulated in an analog computer to give a representation of the phenomena with a quickened time scale. The diagram for the system is given in Figure 1 (BRANSON 1961).

Coefficients of inhibition

The further usefulness of this mathematical model comes to light when we wish to discuss data on the effects of metabolic inhibitions and other chemical agents on the transport of P in normal and sickle cell blood samples. The parameter which comes to

mind is the ratio (x_1/x_0) without the agent and with the agent. We propose, therefore, that the quotient of these two ratios be defined as the coefficient of inhibition, I, thus

$$I = \frac{(x_1/x_0)\ \text{control}}{(x_1/x_0)\ \text{with agent}}$$

Table 3 summarizes the experimental data for the coefficients of inhibition for samples in which sodium fluoride was the metabolic inhibitor. The experimental details are given in LABAT & BRANSON (1958).

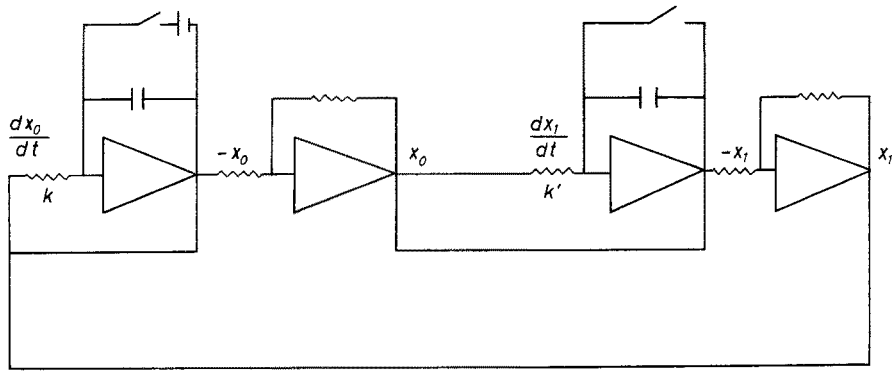

Fig. 1: Diagram for the system in red blood cells. The triangles represent the amplifiers. Different values of k and k' may be tried by changing the input resistors and the feedback capacitors period

Table 3

Results with NaF

Condition	Number of patients	Coefficients of inhibition
Normal	16	1.40–3.88 (2.26 average)
Sickle cell	14	0.86–1.20 (1.05 average)

The impressive aspect of these data is that the maximum value of the coefficient of inhibition in the sickle cell cases is significantly below that of the minimum value for the normals. One conclusion is that whatever mechanism NaF affects in the normal transport mechanism is either inoperative in the sickle cell or protected from the effect of the inhibitor.

CONCLUSIONS

We have shown that a simple mathematical model leads to unequivocal parameters in terms of which the transport and the inhibition of transport of P across the membrane into the interior or the red blood cell in normal and sickle cell samples can be discussed. The model was designed so as to be consistent with the paucity of data

available experimentally, e. g., a three compartment model with the membrane as a compartment yields more parameters but experimentally was not accessible.

SUMMARY

1. A two compartment system for the exchange of inorganic P in the red blood cell and the plasma in blood is worked out in detail.
2. Data on the passage of inorganic P between the plasma and the red blood cell obtained with ^{32}P are interpreted on this model and give 8.65 μg of P as inorganic P per minute in 100 ml of blood while the sickle cell exchange is 10.5 μg per minute.
3. A coefficient of inhibition is defined from the parameters. The data reveal that sodium fluoride inhibits the transport of inorganic P in the normal case but has no effect upon transport in the sickle cell samples.
4. Our interpretation of these results is that the transport system involved is already defective in the sickle cell samples.

ACKNOWLEDGEMENT

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Discussion following the paper by BRANSON

MILLER: (1) Were studies made on red blood cells from patients with sickle cell trait as well as sickle cell anemia? (2) Were sickle cells studied in an atmosphere of nitrogen as well as of oxygen?

BRANSON: We ran a few samples of sickle cell trait blood. The values were intermediate between normal and sickle cell anemic. In an atmosphere of nitrogen we got much sickling in a preliminary experiment; hence we worked only in an atmosphere of oxygen.

AEBI: You mentioned that there might be something like a third compartment in your system. Did you consider that the chemically heterogenous composition of total intracellular ^{32}P-activity (free phosphate + esterified phosphate) may as well explain the data presented? What was the concentration of phosphate-esters in normal red cells and in blood of sickle cell anemia under the conditions you have incubated them?

BRANSON: (1) The reference was to the possibility of describing the data with a three compartment system. Since the third compartment was not accessible, we did not consider it. (2) We examined the phosphorus which is characterized chemically as total phosphorus, soluble phosphorus, and inorganic phosphorus. The low metabolic rate in the red blood cells makes other organic fractions difficult to examine accurately.