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Abstract: Human erythrocytes are highly specialized enucleate cells that are involved in providing efficient gas transport. Erythrocytes have been extensively studied both experimentally and by mathematical modeling in recent years. However, understanding of how aggregation and deformability are regulated is limited. These properties of the erythrocyte are essential for the physiological functioning of the cell. In this work, we propose a novel mathematical model of the molecular system that controls the aggregation and deformability of the erythrocyte. This model is based on the experimental results of previously published studies. Our model suggests fundamentally new mechanisms that regulate aggregation and deformability in a latch-like manner. The results of this work could be used as a general explanation of how the erythrocytes regulate their aggregation and deformability, and are essential in understanding erythrocyte disorders and aging.

Dear Sir or Madam,

RE: Submission of a revised version of our paper “Erythrocyte: a systems model of the control of aggregation and deformability” to *BioSystems*.

We are most grateful to the reviewers for the further detailed and helpful comments on our paper. In our second revised submission we have made a number of further changes to the paper which address the remaining issues raised by the reviewers. A detailed list of these issues (in italics), together with the changes made in the paper to address them (in normal font), is provided below. We hope that our manuscript is now acceptable for publication in *BioSystems*.

Yours sincerely,

Professor Nikolay V Kotov

Reviewer #1

Bazanovas et al. present a hypothesis about regulation of erythrocyte aggregation, deformability, and permeability of the membrane to gases. In short, they suggest that a mechanoreceptor causes a short pulse of intracellular calcium when the erythrocyte enters a capillary. This pulse may switch the cell between two stationary states with different calcium concentration. Intracellular calcium then effects cytoskeleton and aquaporins via a protein kinase signal system with three hypothetical effector proteins. From a few of the components known to be present in erythrocytes and interactions between them they constructed a model which indeed showed two stationary states under certain conditions.
There was only one stationary state when a slightly different set of parameters was used.

I do not think this hypothesis is plausible physiologically (but this is not the argument against publication).

I do not support to publish the manuscript in its present state because of two reasons:

I) citation of literature concerning the gas exchange in erythrocytes is selectively skewed towards a possible role of AQP1, and thus misleading.
In particular:
- establish a relation to Band3 transport, or substantiate, why you believe that aquaporins provide a significant contribution to CO_2 exchange in erythrocytes

> In this study, we consider a general system of regulation of erythrocyte properties. This is a common practice in systems biology modeling papers. We do not focus on specific proteins, such as Band3, but give additional examples of what type of proteins might be included in the scheme. Since the gas exchange is not crucial to the signal system being considered, and has been chosen as an additional property of the erythrocyte, we have removed everything related to the control of gas transport through the membrane of erythrocytes from the paper. Only the control of aggregation and deformability are considered now.

- discuss in more detail why you believe that oxygen transport can be regulated by aquaporin. (While Cooper et al provide a hint that aquaporins might be involved in O_2 transport, textbook knowledge says

that the thickness of the unstirred layer of blood plasma is the main bottleneck to oxygen diffusion. Several investigations around 1980 and before have found that the RBC membrane is no significant barrier to oxygen diffusion - which is compatible both with diffusion through channels and through the lipid phase of the membrane. To my knowledge there are no experiments that found any hint to regulation of oxygen exchange by the RBC membrane)

We agree with the reviewer that the discussion of oxygen transport and the role of aquaporin could be broader. However, there is in fact only one work (Ivanov, I.I., Loktyushkin, A.V., Guskova, R.A., Vasilev, N.S., Fedorov, G.E., Rubin, A.B., 2007. Oxygen channels of erythrocyte membrane. Dokl. Biochem. Biophys. 414,137–140) in which it is shown that O_2 is transported through the AQP1 channels.

- discuss the results in relation to the other attempts to model the state of an erythrocyte

Information about the previous attempts to model the state of the erythrocyte has been added to the main text of the manuscript (Page 4, Introduction). It should be mentioned, however, that although there have recently been several attempts to model erythrocyte states, properties of the erythrocyte such as aggregation and deformability have never been modeled before. We have thus added a discussion of the results in relation to these previously published models.

I recommend that the authors search for a co-author who has a good knowledge of "erythrocyte literature" to achieve a broader and more balanced presentation where the presented hypothesis fits into current knowledge, and where it is highly speculative or in contradiction to established results.

II) presentation is not clear enough
- I suggest to shift eq. 1-23 to the Supplement. These equations are not useful to understand the essence of the paper. The essential features of the model and its implementation should rather be described .- 1..4,

In accordance with the referee’s wishes, we have left three equations in the main text (Page 8) and moved the others to the Supplementary Materials.

@ in Fig. 3 must be explained - all figures should be translated back to "not normalized" values.

This has been amended accordingly. All figures have been translated to unnormalized values.

- re-organize Table 1 and 2, or make clear what it is. In my eyes this is a weird mixture of normalization (shift this to Supplement), Parameters of the model (ok, make clear that this is input), and ranges (I guess this is output, and should better be discussed in the text).

Tables 1 and 2 have been reorganized and shifted to the Supplementary Materials accordingly.

- Table 3 and 4 should also be shifted to the Supplement. I cannot see a direct relation to the results discussed in the text

Tables 3 and 4 have been shifted to the Supplementary Materials section, as requested.

- several figures have to be redrawn to be still readable in b/w after appropriate scaling

All figures have been redrawn to provide better readability.

Reviewer #2

The manuscript entitled "A Systems Model of Control of Aggregation, Deformability, and Permeability of Membranes to Gases" argues that erythrocytes possess a relatively simple signal system, which is coupled to membrane mechanosensitive ion channels and regulates cell aggregation, deformability and membrane permeability.

While the idea put forward in the manuscript is interesting, the authors fail to provide a sound evidence for existence of such a signal system. Example: As the reference for the existence of mechanosensitive Ca++ channels the authors cite a paper Johnson et al. (1994), which only deals with sodium and potassium channels (not calcium), and the paper Brain et al. (2004) where the evidence for the mechanosensitive Ca++ channels is very indirect. If such mechanosensitive Ca++ channels exists, why not demonstrate their existence in experiments, e.g. by micropipette induced mechanical stress?

In the paper of Brain et al. (2004), the labeled calcium indicator Fluo-3/AM was used to measure the calcium influx. It has been shown that, when an erythrocyte is moving through a capillary of diameter 3 μm, the concentration of calcium increases in 30% of cells. It has been shown (Johnson, R.M., Tang, K., 1992. Induction of a Ca^{2+}-activated K+ channel in human erythrocytes by mechanical stress. Biochim. Biophys. Acta. 1107, 314–318) that the Ca^{2+}-activated K^{+}-channel in human erythrocytes works under mechanical stimulation. The paper of Larsen et al. (Larsen FL, Katz S, Roufogalis BD, Brooks DE (1981) Physiological shear stresses enhance the Ca2+ permeability of human erythrocytes. Nature) describes these experiments being performed using radioactive ^{45}Ca, and shows that physiological shear stresses enhance the Ca^{2+} permeability of human erythrocytes. Thus, the results of these papers allowed us to propose the existence of mechanosensitive calcium channels that build the signal system.

Likewise, the possible molecular mechanisms of the relation between Ca++ influx and erythrocyte parameters are described in a very vague way. Many of the proteins involved in erythrocyte deformability

(spectrin?), and membrane permeability have been described in the literature; still the authors refer to them as generic "EF1, EF2 and EF3".

Reviewer #1 has raised a similar point, asking for more details about specific proteins, such as Band3. We would like to point out that considering the generic elements of the scheme and giving specific examples is a common practice in the field. The aim of our study is to show the underlying mechanism of the regulation of erythrocyte properties.

Overall, the paper is written in a way which makes it is very hard to distinguish between facts and speculation. For example, the opening paragraph of Discussion reads "In this work, the behaviour of the signalling system for erythrocytes has been investigated. Based on the analysis of the model, we can show that the parameters of erythrocytes aggregation, deformability, and permeability can switch in a latch-like manner." A naive reader could understand that these are all facts, while it should read instead "If a described system for regulation of aggregation, deformability and membrane permeability exists, it would behave as described in the manuscript."

In our work, we say that the proposed signal system is hypothetical; however, at the same time, we show that it is based on experimental evidence (Pages 5-7, 1.1. The Signal System of erythrocytes). The reviewer is right that it should be clearly stated in the discussion that our results are based on the theoretical analysis of the model, rather than on experiments. The text in the discussion has been amended accordingly (Pages 14-16).

While I'm not an expert in "system's biology" (my expertise is in cellular mechanics) and I can't comment the specifics of the modeling, it seems that the number of model parameters is huge, and that values for many parameters are given without an experimental justification.

The proposed model is based on 7 calcium-calmodulin dependent ferments. The underlying molecular mechanisms of how these ferments work and the relevant parameters are analyzed in (Valeyev et al. 2008a,b). Here we use the results obtained in their works to estimate the parameters.

I can't recommend publication of this manuscript unless the authors provide a sound experimental evidence for the existence of the described signaling system and its underlying molecular mechanisms. The task would be easier if the authors focus to only one of the erythrocyte parameters, e.g. deformability.

The reviewer is asking for sound experimental evidence for the existence of such a signal system. In fact, the existence of the elements included in our signal system, and of the links between them, has been shown experimentally (Pages 5-7, 1.1. The Signal System of erythrocytes). Although not all of the model parameters have been obtained in experiments, they can be assessed quantitatively through the proposed model. If this signal system of erythrocytes exists and is able to switch aggregation and deformability, the chosen parameters should make the system work.

Reviewer #3

Authors present a mathematical model of the erythrocyte describing its assumed signaling system. They found that as the erythrocyte in the capillaries change the ability of erythrocytes to form roleaux and to deform as well as the permeability of the membrane for gases switch from one steady state to another in a latch-like manner with approximately the same thresholds.

Abstract: the authors should briefly describe the aim and scope of the work, the methods used and the results. Presently it is not clear from the abstract that the authors constructed a mathematical model. Please consider that the readers should get all the important information from the abstract.

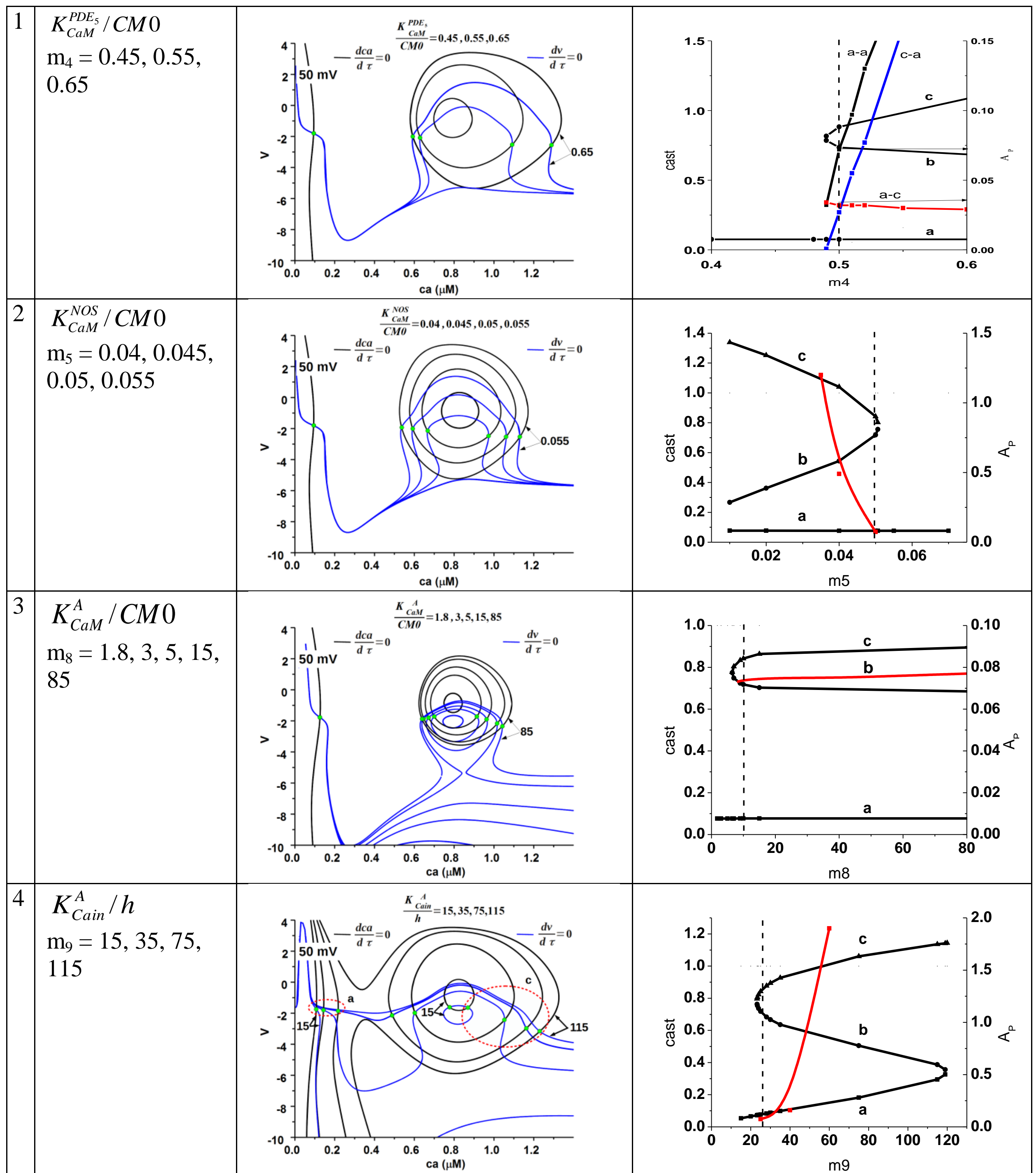
The abstract has been amended in accordance with the referee's wishes.

Mathematical model: Authors construct a sophisticated description taking into account a complex schemes presented in the introduction. They state the model equations and refer to more detaield description in the supplementary material. I am a theoretical physicist but following the text it was difficult for me to recover the derivation of the model equations and relate them to the supplementary material. The authors should offer more instructions to the readers. Please refer to particular equations in the supplement and in the text. Also, equations should not have redundant numbering in order to distinguish between them. The equations in the supplementary material could be numbered as (S1), (S2), etc. The authors should acknowledge that some readers that may take interest in the results are not skilled with mathematical equations so more help and explanation should be offered to understand the results. Fore example in Caption to Fig. 1 they describe the results as »The phase portrait of the system« which is in my opinion obscure. It is only in the last line of the caption that we learn that they present the membrane potential in dependence of the calcium concentration. Please simplify the descriptions.

This has been amended accordingly. The equations in the Supplementary Materials have been numbered as (S1), (S2), etc. The descriptions have also been simplified.

The authors state that the model has 40 parameters for most of which the values are not known. Further they state that they were able to »make our system work«. It is clear that any feature can be described by introducing a sufficient number of adjustable parameters into the system.

All parameters have been analyzed, but only the parameters with the most impact on erythrocyte properties have been included in the paper. As an additional example, the table below shows the influence of 4 parameters that were not included in the manuscript:



The range of possible change in the parameters has been determined. It can be seen from the figures above how many of the singular points are for specific parameter values. The parameter values given in Table 3 in the Supplementary Materials make up one of the possible parameter sets. All the parameters in a certain range have an impact on the shape of the zero isoclines, on the number of singular points, and on the dynamics of the system. We will show this analysis in the next paper.

The authors should clarify in the Discussion and in the Conclusions what is the novelty of their work and justify the effort that an interested reader would spend in comprehending sophisticated model and its

results. A critical analysis presented in a reader-friendly manner revealing which parameters, equations and therefore processes have gross effects and which parameters contributed only the details, would increase the quality of this work.

The Discussion and the Conclusions have been amended in accordance with the referee's wishes.

I believe that theoretical physics and modelling are yet underappreciated in the study of biological systems. These methods have great advantages as they focus the description and modeling towards the relevant features; they are ethically superior as the do not require sacrifice of experimental animals, they are cost-efficient and I believe that they are necessary to better understand the mechanisms. It is therefore of importance that the authors present their valuable work in such manner as to invite also non-physicsts to share the results. I believe that this can be achieved with some additional effort of the authors that is in my opinion worth spending to support the use of mathematical modeling in biology and medicine.

We agree with the referee that the methods used in systems biology might help in understanding the underlying molecular mechanisms. We hope that the changes we have made in the manuscript will make it easier to read and understand the proposed model.

Paper: Erythrocyte: a systems model of the control of aggregation and deformability.

Antonina N. Bazanovas[1], Aleksandr I. Evstifeev[1], Ildar I. Sadreev[2], Andrey I. Skorinkin[1,3,4], Nikolay V. Kotov[1]

Highlights

1) In this study we employ the techniques of structural systems biology to investigate the mechanisms of regulation of erythrocyte properties. We build a mathematical model of regulation of erythrocyte aggregation and deformability. The model is based on the experimental facts from previously published papers.
2) Our model suggests that when moving along the bloodstream, the erythrocyte switches its aggregation and deformability from one steady state to another in a latch-like manner.
3) The proposed model allows us to extend the understanding of erythrocyte phosphorylation-regulated circuits and mechanisms of regulation of its aggregation and deformability. Our model predictions are consistent with the experimental observations published in the literature before. The obtained results are significant in many erythrocyte pathologies.

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Erythrocyte: a systems model of the control of aggregation and deformability

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Abstract

Human erythrocytes are highly specialized enucleate cells that are involved in providing efficient gas transport. Erythrocytes have been extensively studied both experimentally and by mathematical modeling in recent years. However, understanding of how aggregation and

deformability are regulated is limited. These properties of the erythrocyte are essential for the physiological functioning of the cell. In this work, we propose a novel mathematical model of the molecular system that controls the aggregation and deformability of the erythrocyte. This model is based on the experimental results of previously published studies. Our model suggests fundamentally new mechanisms that regulate aggregation and deformability in a latch-like manner. The results of this work could be used as a general explanation of how the erythrocytes regulate their aggregation and deformability, and are essential in understanding erythrocyte disorders and aging.

Keywords

Erythrocyte; Calcium; Calmodulin; Aggregation; Deformability.

Abbreviations

SS	The signal system that regulates the parameters of the erythrocytes.
AG	The ability to form rouleaux — aggregation.
D	The ability to change their shape under action of external forces - deformability.
EF1, EF2	proteins, phosphorylation of which leads to changing of the AG and D.
CaM	Calmodulin.
AQP1	Aquaporin 1.
cAMP	Cyclic 3˙,5′ adenosine monophosphate.
cGMP	Cyclic 3˙,5′ guanosine monophosphate.
NO	Nitrogen monoxide.
GC	Guaylate cyclase.
AC	Adenylate cyclase.
PKA	cAMP-dependent kinase.

PKG	cGMP-dependent kinase.
PDEs	Phosphodiesterase.
NOS	Molecular module that regulates concentration of NO.
ACII	Molecular module that regulates concentration of cAMP.
sGC	Molecular module that regulates concentration of cGMP.
ATP	Adenosine triphosphate.

1. Introduction

Erythrocytes are the elements of the complicated multifunctional system, a function of which is maintaining the concentration of oxygen and carbon dioxide in tissues in a given range of values in accordance with the needs of metabolism. This is mainly provided by the intensity of ventilation, the regulation of the heart working, pre-capillary sphincters, and sphincters of arterio arteriovenous anastomoses. When autoregulating the brain's bloodstream, maintaining the concentration is provided by control of the cerebral arterioles (Kamkin and Kamenskiji, 2004). Moreover, it has been shown that the parameters of erythrocytes can change: the ability to form rouleaux — aggregation (AG), the ability to change their shape under action of external forces — deformability (D) (Bishop et al., 2001; Cicco and Pirrelli, 1999; Das et al., 2007; Kim

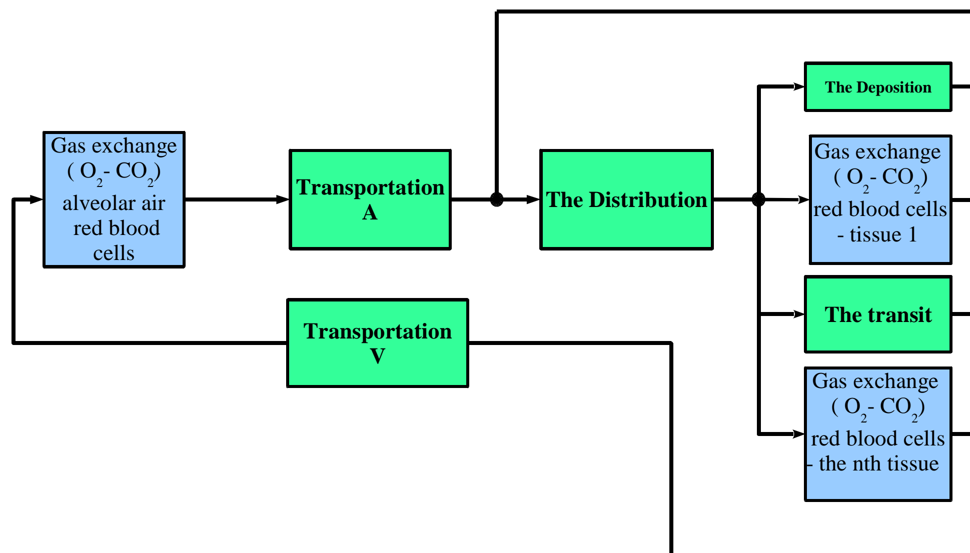

Fig. 1. Functional diagram for the system of bloodstream that describes the erythrocyte mediated breath.

The decreasing of aggregation, increasing of deformability.

et al., 2005; Meiselman, 2009). This also may affect the gas transmission function.

Due to their simplicity, erythrocytes have been a subject of mathematical models in a number of works on systems biology. Early studies captured only the glycolytic pathway (Rapoport et al., 1976). Then the model was expanded to include the pentose phosphate pathway (Ataullakhanov et al., 1981). The first sophisticated model included the sodium–potassium pump and membrane transport (Joshi and Palsson, 1989). Using that study as a basis, and introducing glutathione synthesis and export systems, a simulation analysis of Glucise-6-Phosphate Dehydrogenase (G6PDH). Deficiency has also been developed (Nanda et al., 2013). These mathematical models are good examples of attempts to model human erythrocyte metabolism, but do not cover some properties of erythrocytes, such as aggregation and deformability. In the present study, we introduce a novel mathematical model that suggests possible mechanisms of regulation of the aggregation and deformability of erythrocytes.

Based on an analysis of the mathematical model of the SS, we here suggest that the SS of the erythrocyte not only changes stationary levels of AG and D, but that this SS also switches the parameters of the erythrocyte from one steady level to another, in a latch-like manner. In exchange capillaries there is a high level of D while AG is low; in the other parts of circulatory system there is a low level of D while AG is high. Due to this, there are additional opportunities to optimise the working of the respiratory system (Fig. 1), which allow minimizing the energy spent on moving the blood through the capillaries, minimizing the gas exchange with the walls of blood vessels, and at the same time facilitating the necessary rate of gas exchange in capillaries.

The process of combining erythrocytes into AG is sophisticated and multifactorial and has a significant impact on the main oxygen transport function of the blood (Levtov et al., 1982). AG facilitates the axial drift of the red blood cells and the formation of plasma sheet boundary level (Cokelet and Goldsmith, 1991; Goldsmith et al., 1999). The increased axial accumulation level of red blood cells reduces the local viscosity in the wall zone of the vessel (Alonso et al., 1993; Suzuki et al., 1996) and thereby modulating the activity of the vascular regulatory mechanisms (Baskurt and Meiselman, 2007) and reducing oxygen release to the vessel walls (Tateishi et al., 2002, 2001).

At the level of the exchange capillaries the efficiency of bloodstream depends on D and AG (Bishop et al., 2004, 2001; Das et al., 2007; Kim et al., 2005; Meiselman, 2009; Suzuki et al., 1996), and it increases dramatically for increasing D, and diminishing AG. Fig. 2 shows the functional scheme for the system that controls AG and D of the erythrocytes. The scheme shows

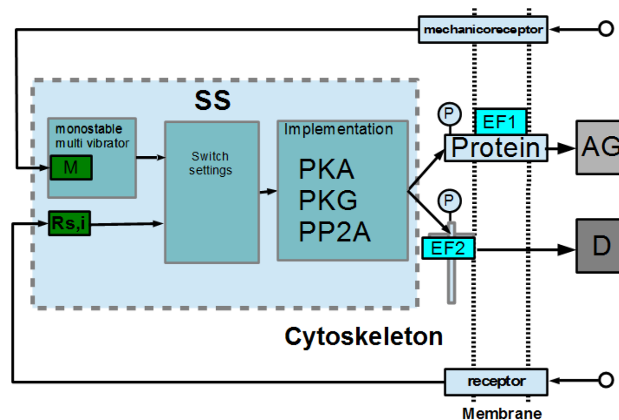

Fig. 2. Functional diagram for the SS of the erythrocyte. M — mechanoreceptor, Rs,i — receptors of ligands that control the parameters of adenylate cyclase. Monostable multi vibrator — it have only one stabl state, and produce single output pulse when it is triggered externally. Swith setings — is a bistable multivibrator that has two stable states. SS — signal system. Implementation — EF1, EF2 — proteins, phosphorylation of which leads to changing of the AG, D.

that the signals from the receptors come to the SS and are then analysed. As a result the control actions are generated, which can change AG and D by phosphorylation of the membrane proteins and the protein of the cytoskeleton. Moreover, under the influence of signals, both stationary changing of AG and D and their latch-like switching may occur.

1.1. The Signal System of erythrocytes

Fig. 3 illustrates the scheme of physic-chemical processes, which take place in the SS of erythrocytes that controls phosphorylation of EF1, EF2. The latter defines the level of AG, D. As mentioned before, the greater the stationary level of phosphorylation of these proteins the less the stationary levels of AG and the higher the level of D (Saldanha et al., 2007). Whether this SS can switch these parameters in latch-like manner is still unknown.

The elements of the SS are physic-chemical processes happening due to the work of certain proteins. Let us consider them in detail.

It has been shown that erythrocytes have membrane proteins AQP1 — aquaporin of type 1 — highly selective water channels (Preston and Agre, 1991). Water, oxygen, and carbon dioxide can move through these channels (Cooper et al., 2002; Endeward et al., 2006; Ivanov et al., 2007; Smith and Agre, 1991), and they are not permeable to charged molecules. When forming the complex of AQP1 with cyclic monophosphates, particularly with cGMP, AQP1 become permeable to cations. Their permeability weakly depends on the membrane potentials (Anthony et al., 2000).

There are several types of calcium channels in the membrane of erythrocytes: potential-dependent I_{ca} (Bennekou et al., 2003; Kaestner, 2011; Soldati et al., 1997) and calcium-calmodulin-dependent channels of active transport of calcium I_{CaA} (Fidalgo da Silva et al., 2006; Zidek et al., 1992). There are also mechanoactivated I_{camec} cationic channels (Brain et al., 2004; Johnson, 1994; Larsen et al., 1981), the main penetrating ion of which is calcium, since its

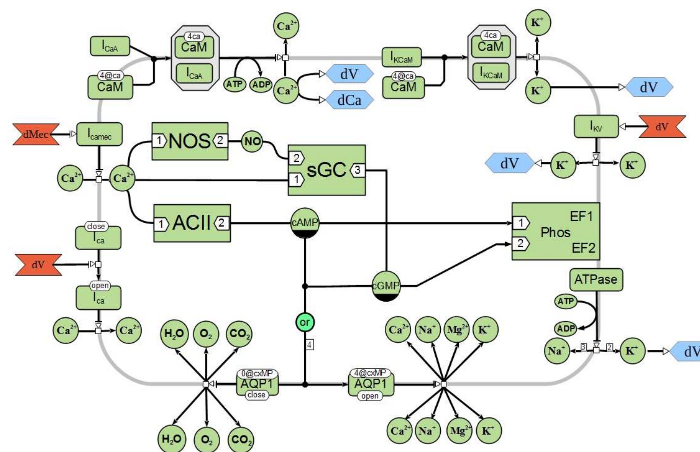

Fig. 3. Schematic diagram for the SS that regulates the level of phosphorylation of the membrane proteins EF1 and the proteins of the cytoskeleton EF2 that regulate AG and D. Ca, Na, Mg, K — ions of calcium, sodium, magnesium, and potassium, respectively. CaM — calmodulin. cAMP — cyclic adenosine monophosphate. cGMP — cyclic guanidine monophosphate. NOS — molecular module that regulates concentration of NO. ACII — molecular module that regulates concentration of cAMP. sGC — molecular module that regulates concentration of cGMP. Phos (EF1, EF2) — molecular module that phosphorylates proteins EF1 and EF2 the level of phosphorylation of the latter defines AG, D. ICaA — channels of active transport of calcium. Icamec — mechanoactive cationic channels. Ica — potential - activated calcium channels. IKcaM — calcium-calmodulin dependent potassium channels. I_{KV} — potential-dependent potassium channels. AQP1 — aquaporins.

electrochemical potential is much higher than that of other cations.

There are also calcium-dependent potassium channels I_{KcaM} in the membrane of erythrocytes (Bennekou et al., 2003; Dyrda et al., 2010; Huber, 2013; Johnson and Tang, 1992), potential-dependent potassium channels I_{KV} (Zidek et al., 1992).

There is a protein calmodulin (CaM) in erythrocytes (Nelson et al., 1983) and the processes, due to which changes of the concentration of cyclic monophosphates (cAMP, cGMP) occur. There are also ferments of their metabolism (Adderley et al., 2011; Bor-Kucukatay et al., 2003; Horga et al., 2000; Kleinbongard et al., 2006; Muravyov and Tikhomirova, 2012, 2013; Petrov et al., 1998, 1994).

Changes in the concentration of cyclic monophosphates modulate the activity of kinases: cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG). These kinases and phosphatase PP2A define the phosphorylation level of the membrane proteins EF1 and cytoskeleton EF2. Phosphorylation of certain proteins affects the rheological properties of blood due to changes of AG and D (de Oliveira et al., 2008; Saldanha et al., 2007).

Fig. 3 illustrates the scheme for the SS that controls phosphorylation of the membrane proteins and the cytoskeleton of the erythrocyte, which in turn determines AG and D. This scheme is built using the SBGN language (Le Novère et al., 2009) taking into account its rules of representing diagrams. Let us to analyse the work of this signal system.

2. Material and methods

We built a mathematical model based on Fig. 3 that contains only two ordinary differential equations: one for the rate of calcium concentration change in the cytoplasm of the erythrocyte $dca\ /\ d\tau$ and one for the rate of change the membrane potential $dv\ /\ d\tau$.

We used steady-state solutions for the other variables (all reactions of complex formation, change of the concentration of NO, cAMP, cGMP) included in our model assuming that their rates of coming to the steady-state exceed the rates of coming to the steady-state for concentration of calcium **ca** and the membrane potential **v**.

As the initial conditions, in one case, we took the values of variables in the singular points, in the other case, we took the values located on the edges of the right half of the quadrant $0.01\ \mu M < ca < 1.2\ \mu M, -200\ mV < v < 50\ mV$.

The scheme shown on Fig. 3 can be written in dimensionless form (for the dimensional form of these equations and details of moving to the dimensionless form see Supplementary Materials) for the rates of change of the calcium concentration, membrane potential of the erythrocyte (1) and the level of phosphorylation of EF1 and EF2 proteins, which define AG and D (2):

$$\begin{cases} \frac{dca}{d\tau} = w_0 \cdot \left(i_{ca}^{mec} + i_{ca}^{L} + i_{ca}^{AQP1} + i_{ca}^{A}\right), \\ \frac{dv}{d\tau} = w_1 \cdot \left(i_{ca}^{mec} + i_{ca}^{L} + i_{ca}^{AQP1} + i_{ca}^{A} + i_{K}^{CaM} + i_{K}^{V} + i^{ATPase}\right), \end{cases} \tag{1}$$

$$\frac{da_p}{d\tau} = h_2 \cdot \left(h_{10} \cdot \frac{pka(ca)\cdot(1-a_p)}{\lambda_{11}+1-a_p} + h_{10} \cdot \frac{pkg(ca)\cdot(1-a_p)}{\lambda_{21}+1-a_p} - \frac{a_p}{\lambda_2+a_p}\right), \tag{2}$$

where $ca = [Ca^{2+}]/h$, $h = 10^{-6}$M , $[Ca^{2+}]$ — concentration of calcium, $\tau = t \cdot r$, $r = 10^3$ c^{-1}, i_j^m — ionic currents, a_P — phosphorylated protein (EF1, EF2). $v = V_m Fz/RT$, V_m — membrane potential, F — Faraday constant, T — temperature, R — universal gas constant, z — dimensionless charge of ion.

The derivation of expressions for the currents i_j^m and for the activities of the ferments as a function of calcium concentration can be found in the Supplementary Materials. We use one equation to describe the phosphorylation of the EF1 and EF2 proteins, since both are phosphorylated and dephosphorylated by the same PKA, PKG, and PP2A. It can be concluded that AG decreases and D increases with the level of phosphorylation of these proteins.

For simplicity, the calcium currents, which under the irritation of the erythrocyte are rapidly inactivated. The mechanoactivated and potential-dependent calcium currents $i_{Ca}^{mec} + i_{Ca}^{L}$ have been presented as rectangular impulses of length $T = t_c - t_0$ and of dimensionless magnitude A, being the change in conductance of the erythrocyte membrane for calcium ions.

$$i_{ca}(\tau) = i_{ca}^{mec} + i_{ca}^{L} = if(t > t_0 \vee t < t_c, A \cdot m(ca), 0) + A_{con} \cdot m(ca), \tag{3}$$

where $m(q) = v - \frac{1}{z} ln\left(\frac{[q_{out}]}{[q_{in}]}\right)$, $[q_{out}], [q_{in}]$ — concentrations of the ion q inside and outside the erythrocyte, respectively.

This function also includes the constant A_{con} , which denotes the change in conductance for calcium ions.

Our model contains parameters, whose values can be found in Tables 1–4 of the Supplementary Materials. We have found the values of the parameters that make our system work in a latch-like manner with two steady-state calcium concentrations: the first under 10^{-7}M, the other under 10^{-6}M. The membrane potential is about -50 mV. The switching from one steady-state to the other and back is induced by similar calcium impulses.

3. Results

3.1 The analysis of the mathematical model

When varying the parameters in a wide range of values, equation (1) gives three fundamentally different types of phase portraits:

1) one singular point (stable node) for calcium concentration $\sim 10^{-7}M$ $(ca \approx 0.1)$ (Fig. 4A);

2) three singular points (two stable nodes): the first singular point for calcium concentration $\sim 10^{-7}M$ $(ca \approx 0.1)$, the second singular point for $\sim 10^{-6}M$ $(ca \approx 1)$ (Fig. 4B); and

3) one singular point (stable node) for calcium concentration $\sim 10^{-6}M$ $(ca \approx 1)$ (Fig. 4C).

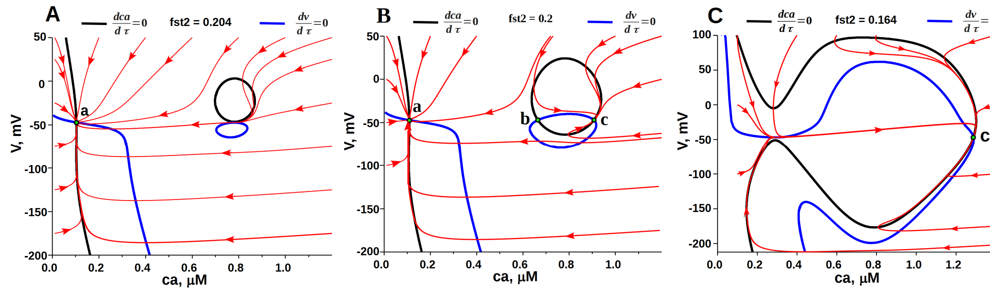

Fig. 4. Phase portraits (red lines) and null isoclines (black and blue lines) of the system (1) for three different parameter values fst2: (A) one singular point a — stable node for the range of low calcium concentrations for parameter value fst2 = 0.204, (B) three singular points a, b, c, where a, c — stable nodes parameter value fst2 = 0.2, (C) one singular point c — stable node for the range of intermediate physiological calcium concentrations for parameter value fst2 = 0.164. V_m — the membrane potential, ca — calcium concentration in erythrocyte.

We analyze next the work of the SS for the parameters that give three singular points **a, b,** and **c**. It can be shown in this case that switching from the **a** to **c** and back may occur by rectangular impulse of calcium current of certain magnitude A and duration T.

Fig. 5 illustrates the family of phase portraits of system (1) for different values of A_{con}. The values of the other parameters are shown in Tab. 1, 2, 3, 4 (Supplement).

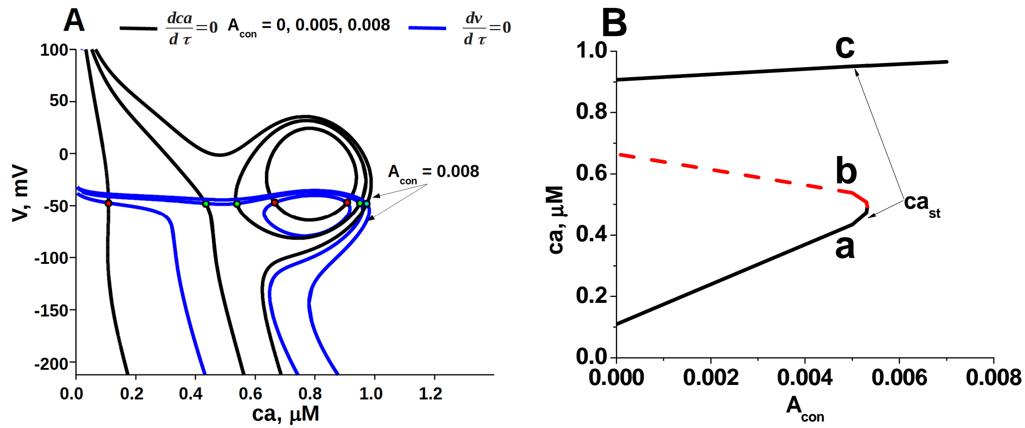

Fig. 5. (A) Zero-isoclines of the system (1) for different values of the parameter A_{con}. V – the membrane potential, ca – calcium concentration in erythrocyte. (B) Stationary concentrations of calcium ca_{st} (a, b, c) as a function of A_{con}, stationary membrane permeability for calcium. The values of other parameters are presented in Tables S1, S2, S3, S4 (Supplement).

It can be seen that for some critical value of parameter A_{con} the change in the number of singular points occur. When $A_{con} \geq 0.011$ system (1) has only the one singular point **c**. For lower values of A_{con} there are three singular points. If initially the system is in **a** (for $A_{con} = 0$), then for $A_{con} \geq 0.011$ the representative point moves to the only one singular point **c**.

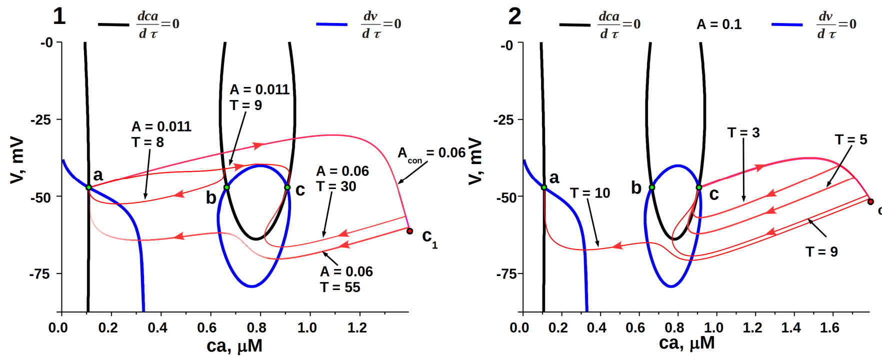

*Fig. 6. The phase trajectories of the system (1) in response to rectangular impulse of calcium current of magnitude A and duration T. (1) Initial values in the state a. Phase trajectories in response to rectangular impulse of magnitude A = 0.011, and durations T = 8, 9; magnitude A = 0.06, and durations T = 30, 55. The pink line represents phase trajectory for A = 0, A_{con} = 0.06 that passes through **a** and a singular point c_1. (2) Initial values in point c. Phase trajectories in response to stimulation by rectangular impulse of magnitude A = 0.1 and durations T = 3, 5, 9, and 10. The pink line represents phase trajectory for A = 0, A_{con} = 0.1 that passes through **c** and a singular point $\mathbf{c_1}$. V - the membrane potential, ca - calcium concentration in erythrocyte.*

Depending on the impulse duration T, the behavior of the representative point may be different. Fig. 6 shows phase trajectories of model (1) in response to rectangular impulses of a magnitude A and duration T.

The switching from a singular point **a** to **c** is caused by the rectangular impulse of the magnitude $A = A_{con}$, at which system (1) has only one singular point c_1 (Fig. 4C) and the duration of the impulse is in a certain range. In this case the representative point, which initially was in **a**, when under the action of impulse of the magnitude A, starts to move to a singular point c_1. (This phase trajectory is marked by pink colour.) Then, depending on the impulse duration, the representative point may come back to the singular point **a** (Fig. 6(1), 7) or switch to a singular point **c** (Fig. 6(1), 7(1), $A = 0.011$, $T > 9$), or pass the singular point **c** and return to a singular point **a** (Fig. 6(1), 7(1), $A = 0.06$, $T \geq 55$).

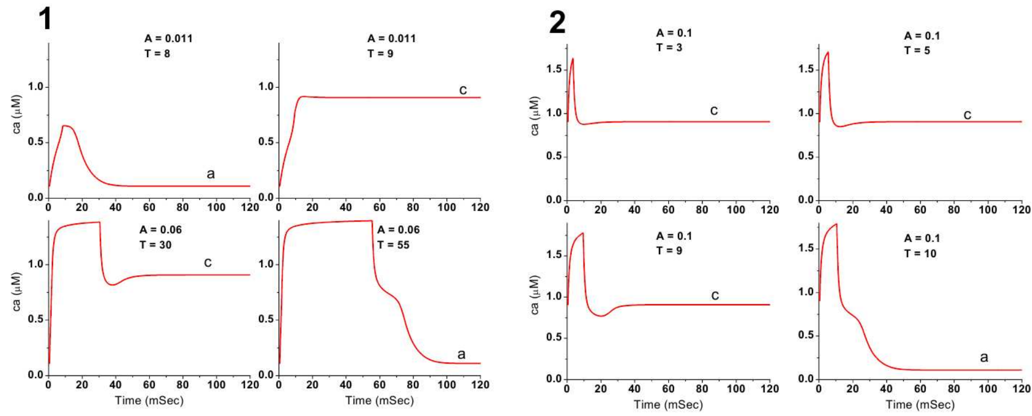

*Fig. 7. The concentration of calcium (ca) as a function of time when the cell is stimulated by the rectangular impulse of magnitude A and duration T. The impulse is turned on when t = 4. The duration of the impulse is denoted by double dotted lines. (1) Initial values in singular point **a**. The cell undergoes impulses of magnitude A = 0.011, and durations T = 8, 9; magnitude A = 0.06, and durations T = 30, 55. (2) Initial values in singular point **c**. The cell undergoes impulses of magnitude A = 0.1 and durations T = 3, 5.30, 55.*

The switching from a singular point **c** to **a** is caused by the rectangular impulse of the magnitude $A = A_{con}$, at which the representative point of system (1) by the end of action of the impulse, is in the phase trajectory (Fig. 6(2), 7(2), $A = 0.1$, $T \geq 10$), which moves to a singular point **a**.

In response to the rectangular impulse of certain magnitude A and duration T, our model switches from a steady-state **a** to a steady-state **c** and vice versa. Fig. 8 illustrates the threshold characteristics of our system. If the parameters of the impulse are in the red field, then the erythrocyte switches from a singular point **a** to **c**, and from **c** to **a**.

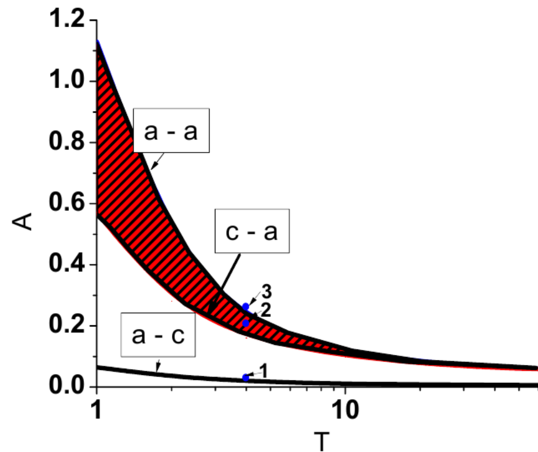

Fig. 8. Division of impulse space into regions representing different reactions of system (1). The line a-c: if the magnitude A of stimulating impulses is higher than the line (a-c) at time T the system undergoes from state a to state c. The line c-a: if the magnitude A of stimulating impulses is higher than the line (c-a) at time T the system undergoes from state c to state a. The line a-a: if the magnitude A of stimulating impulses is higher than the line (a-a) at time T the system returns from state a to state a. The region marked by red colour represents the set of parameters of stimulating impulses, for which the system undergoes from the a to c and from the c to a. Points 1, 2, 3 show the parameters of the impulses for calcium time dependent characteristics shown in Fig. 9.

Fig. 9 illustrates calcium concentration in the erythrocyte as a function of time when

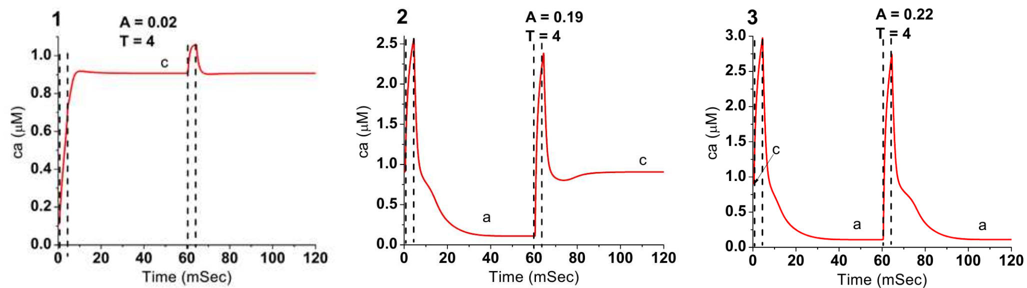

Fig. 9. The concentration of calcium (ca) as a function of time when the cell is stimulated by the two rectangular successive identical impulses, the parameters of which are in (1) a to c, c to c (point 1 in Fig. 8), (2)c to a, a to c (point 2 in Fig. 8), (3) c to a, a to a (point 3 in Fig. 8). The dash lines indicate the moment of action of the impulses.

stimulating the cell by the impulses, the parameters of which are in different fields. There are three blue points 1, 2, 3 belonging to a certain field (Fig. 8).

So what determines the amplitude and duration of calcium impulses, which are run by mechanoreceptors? On the one hand the speed of erythrocytes movement when entering the pre-capillary area does it. This speed determines time, during which the erythrocyte is being deformed (impulse duration). On the other hand the diameter of pre-capillaries, which determines how much the erythrocyte is deformed (magnitude of the impulse). If we take into account potential-dependent channels then the dependence of parameters of calcium impulse will also be determined by the characteristics of the channels.

We have found the parameters (Table S3), at which system (1) has three singular points **a, b, c**, located on the axis of potential in the area -2 (-50 mV), on the axis of calcium concentration **a** ($ca \approx 0.1\ \mu M$), **c** ($ca \approx 1\ \mu M$). Moreover, we have found the parameters, at which the system switches from **a** to **c** and vice versa in a switch-like manner (the threshold characteristics for **a** and **c** are almost equal). From our point of view these parameter values of the SS of erythrocyte may be determined as one of the normal variants.

However, it is worth to note that all the parameters of real erythrocytes (channel density, total concentration of ferments etc.) are continuously changed.

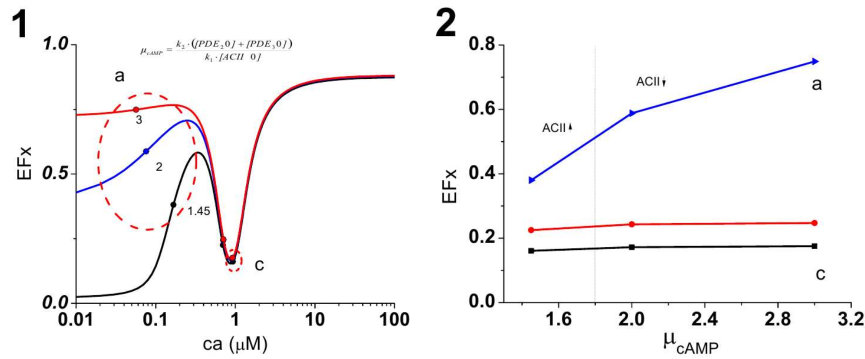

Fig. 10. (1) The levels of non-phosphorylated proteins as a function of the concentration of calcium for different μ_{cAMP}. (2) The dependence of the stationary level of non-phosphorylated proteins as a function of the parameter μ_{cAMP}. (a, c) is singular point. $\mu_{cAMP} = \frac{k_2 \cdot ([PDE_2 0] + [PDE_3 0])}{k_1 \cdot [ACII0]}$.

Erythrocyte does not have nucleus, endoplasmic reticulum and therefore system of protein regeneration. Different proteins have different $t_{1/2}$: from several hours to several months. Thus, real erythrocytes have a discrepancy of all parameters, which lead to different steady-state

calcium concentrations and thresholds. How does this affect their dynamic behavior depends on parameters of the discrepancy.

Under the action of various pathologies, aging and physiologically active compounds the changes in the parameters of the SS take place. The situations are possible where there is only one singular point – either **a** or **c**. In this case, the system responds only by pulse change of its characteristics in response to calcium pulse stimulation.

A special case of pathology are such changes of the parameters of our system, in which there are three singular points, but at the same time there are great differences between thresholds of switching from **a** to **c** and from **c** to **a**, which may lead to high discrepancy of steady states of erythrocytes under various conditions (under various speeds of bloodstream).

3.2 Protein Phosphorylation

As it was mentioned before, erythrocyte has kinases and phosphatase that are responsible for the phosphorylation of EF1 and EF2 proteins, which are responsible for the AG, D. Fig. 10 illustrates level non-phosphorylated proteins EFx as a function of calcium concentration in the erythrocyte for the following parameters $\mu_{cAMP} = 1.45, 2, 3$. It can be seen from the figures that μ_{cAMP} decreases when the activity of ACII increases. At the same time, the aggregation decreases and the stationary calcium concentration increases.

4. Discussion and conclusion

In this work, we propose a mathematical model of the signal system that regulates properties of the erythrocyte such as aggregation and deformability. The proposed model is consistent with experimental studies on erythrocytes. The results we obtain show that the parameters of erythrocyte aggregation and deformability can switch in a latch-like manner.

As it was mentioned the presence of molecules (Fig. 3) and links between them, which are included into the SS of erythrocyte, have been shown experimentally. In addition to these molecules, which could be included into the SS, there are others that we have not yet considered. However, not all of the properties of these molecules are revealed in experiments. Total protein concentrations, specific gravities of channels and their characteristics, enzymatic reactions rate constants, Michaelis constants, levels of calcium-calmodulin independent activities of enzymes and ion channels have been evaluated theoretically. Our theoretical evaluations do not exceed physiological limits. The model predicts that the erythrocyte is able to switch in a latch-like manner not only for the used set of parameters. Many parameters are interrelated, when a change

in one may be compensated by changes in the other. Finding the limits of how the parameters may change with regard to their mutual influence, in which the erythrocyte is normally functioning, requires a new study.

Beside the fact that there are many proteins forming the SS in erythrocyte, which can work in a latch-like manner, there are some other facts that support the existence of the proposed SS. Erythrocytes have mechanoreceptors that are able to increase the permeability for calcium and other cations under mechanic stimulation. In the work (Brain et al., 2004) it has been shown that the concentration of calcium in all erythrocytes initially is approximately 10^{-7}M. When the erythrocyte is moving through the capillary, the diameter of which is 3-5 microns (less than the diameter of erythrocyte), 30% of erythrocytes have calcium concentration approximately 10^{-6}M while the others 10^{-7}M. Our mathematical model suggests that dysfunctions in the proposed SS may lead to this effect.

It is currently not clear how to explain the increase of calcium concentration in order in only 30% of erythrocytes.

If we assume that the SS (Fig. 3) exists, then this result can be interpreted in such a way that, upon entry into a capillary, the concentration of calcium in many of the erythrocytes will increase up to 10^{-6} M; however, on leaving the capillary and undergoing repeated mechanical stimulation, the calcium level returns to its initial value in only 70% of erythrocytes in the blood sample (Brain et al., 2004). In other words, 70% of erythrocytes have such values of parameters and the rate of moving through the capillary, that they at the first mechanical stimulation move from the steady-state **a** to **c**, and at the second stimulation return back (Fig. 12). It should be noted here that 30% of erythrocytes after moving to steady-state **c** under the action of the second pulse remain in **c**. This fact is considered as an indirect evidence of the existence of the SS discussed in this study.

It has been shown that the aging of erythrocytes is accompanied by the increasing of their calcium concentration (Aiken et al., 1992). For young erythrocytes it is in average $0.62 \bullet 10^{-7}$M, while for the olds it is approximately $2 \bullet 10^{-7}$M, but this all is for the field of the steady-state singular point **a** (Fig. 4B). Therefore it can be assumed that during the aging of the erythrocyte the system of calcium homeostasis deteriorates.

It has been shown that both the condition of certain membrane proteins and the concentration of certain plasma proteins covering the cell surface affect the aggregation of erythrocytes.

We assume here that the spread of erythrocytes over the states with different levels of phosphorylation of protein Efx (in singular points **a** and **c**) also affects the aggregation. The erythrocytes that are in **c** facilitate stopping of growth of the rouleaux since there is a low aggregation in this state. The more erythrocytes are in state **c**, where they are moving through large capillaries, the less is the average length of the rouleaux. When all the erythrocytes are in **c** both the rouleaux and erythrocyte sedimentation rate are the smallest.

This study suggests new mechanisms for the regulation of aggregation and deformability of the erythrocyte. The analysis of the proposed model demonstrates the conditions and parameters values under which the erythrocyte switches its aggregation and deformability from one steady state to another, in a latch-like manner with approximately the same thresholds. The proposed mathematical model may be useful in understanding the mechanisms of erythrocyte diseases and aging.

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Figure

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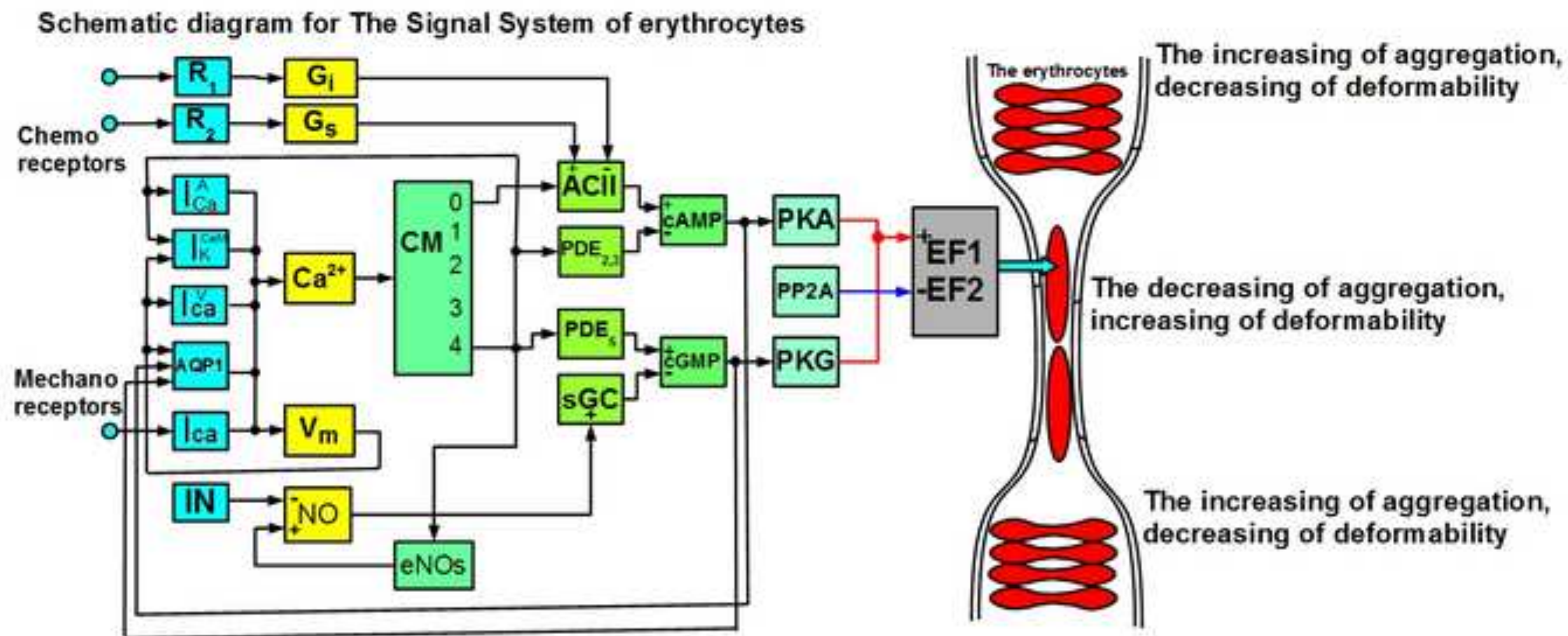

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