# Relationship between $H^+$ Transfer through Human Erythrocyte Membrane and Temperature

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The parameters of thermal dependence of $H^+$ transport into human erythrocytes in the presence of furosemide were measured. The relationship between these changes and function of band 3 protein of the erythrocyte membrane is discussed.

**Key Words:** *$H^+$ transport; Jacobs—Stewart cycle; furosemide; band 3 protein*

$H^+$ transport through membranes of cell and organelles plays an important role in the regulation of pH [8]. Investigation of this problem is particularly important for erythrocytes, because respiratory function of the blood depends on pH [2]. $H^+$ transport through erythrocyte membranes is realized during the Jacobs—Stewart cycle reactions [7] with involvement of capnoforine (transmembrane band 3 protein, b3), which realizes the $HCO_3^-/Cl^-$ metabolism and plays an important role in the regulation of $CO_2$ transport in the blood [6].

Under natural conditions the extracellular noncatalyzed reactions of the Jacobs—Stewart cycle are the limiting stages of $H^+$ transport through the membrane, because the rate of these reactions is lower than the rate of b3 work [3]. Under experimental conditions changes in b3 working in response to certain exposure, rather than its absolute values are important, and therefore inhibitors of anion exchange can be used to reduce this rate so that it became the limiting step in $H^+$ transport. In our experiments we used furosemide (a wide-spectrum drug) [5,10,11].

We analyzed specific features of transmembrane transfer of $H^+$ under different thermal conditions, because it is known that erythrocytes realizing $CO_2$ and $O_2$ transport work in different vascular system areas under different thermal conditions.

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## MATERIALS AND METHODS

Adult donor blood was centrifuged at 3000 rpm for 8 min; the supernatant was discarded and the erythrocytes were washed in 0.9% NaCl (pH 7.4) and recentrifuged under the same conditions. Compact precipitate of erythrocytes (100 μl) was added to 10 ml of 0.9% NaCl solution. Furosemide in a concentration of 0.05% was added to the samples. The rate of $H^+$ transport was evaluated by the standard method [4] at 10, 15, 20, and 25°C. The relationship between the rate of this process and temperature was characterized by thermal coefficient ($Q_{10}$) and activation energy ($E_a$), which was calculated by the formula:

$$E_a=RT_1T_2(\ln k_2-\ln k_1)/(T_2-T_1),$$

where $k_1$ and $k_2$ are constants of $H^+$ transport reactions activity at $T_1$ and $T_2$ temperatures, respectively, and $R$ is Boltzman constant (8.31 J/mol×K).

## RESULTS

The rate of changes in the pH of the suspension (the criterion of $H^+$ transport into erythrocytes) increased with increasing the temperature (Fig. 1): the slope of the curve corresponding to the 1st min of the experiment increased and the initial points of pH were shifted upwards.

Three intervals can be distinguished on the curve, where the rate of pH changes was calculated as

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*dpH/dt*: 1) 10-15°C: negligible acceleration of $H^+$ transport; 2) 15-20°C: appreciable changes in $H^+$ transport rate; and 3) 20-25°C (Fig. 2). For the 1st interval $Q_{10}$=1.8, $E_a$=18 kCal; for the 2nd interval $Q_{10}$=3.6, $E_a$=9.4 kCal; and for the 3rd interval $Q_{10}$=1.5, $E_a$=23 kCal. The constants for intervals of 10-15 and 20-25°C in our experiments coincide with previous findings [9]. This previous study [9] was carried out with high concentrations of carbonic-anhydrase and $HCO_3^-$, that is, under conditions when (similarly as in our experiments) the rate of $H^+$ transport into erythrocytes depended on the rate of b3 work. The method used in our study helped us to evaluate adequately the temperature dependence of anion exchange. Presumably, it can be used for evaluation of other parameters of b3 work.

Appreciable changes in $Q_{10}$ and $E_a$ between 15 and 20°C attest to deep changes in the erythrocyte membrane in this thermal interval. These thermodynamic parameters carry no structural information, but they depend on the physicochemical characteristics of lipid molecules, determined by phasic transitions in the membrane [1]. It is noteworthy that as the velocity of $H^+$ transport increased with increasing the temperature, hemoglobin in the erythrocyte experienced more and more intense effects of $H^+$, and as we know [2], this is paralleled by a decrease of hemoglobin affinity for $O_2$.

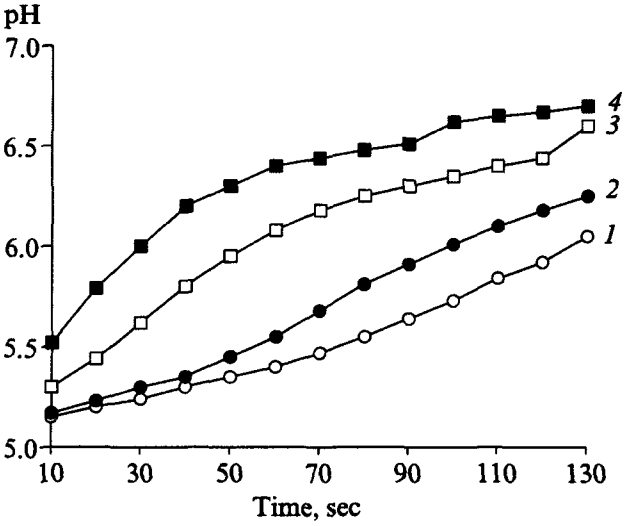


**Fig. 1.** Dynamics of pH in human erythrocyte suspension at different temperatures after acidification. *1*) 10°C; *2*) 15°C; *3*) 20°C; and *4*) 25°C.

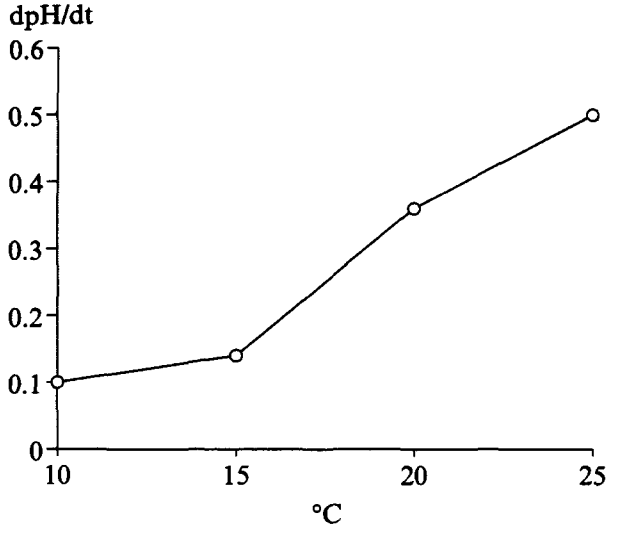


**Fig. 2.** Thermal dependence of the rate of $H^+$ transport into human erythrocytes.

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