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IONIC COMPOSITION AND POTASSIUM PERMEABILITY OF ERYTHROCYTE MEMBRANES
FROM PATIENTS WITH THE INFLAMMATORY-DYSTROPHIC FORM OF PERIODONTOSIS

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During the development of pathological processes considerable disturbances of acid-base balance in the tissues and of the ionic composition of the erythrocytes take place [1, 5, 7, 8]. In periodontosis, a stomatologic disease with most uncertain etiology and one which is most difficult to treat, energy metabolism in the tissues of the mouth undergo substantial changes [2, 3, 6]. One cause of the disturbance of function and energy metabolism of the tissues, it can be tentatively suggested, is a change in the ionic permeability of biomembranes and in the ionic homeostasis of the cells.

Accordingly it was decided to make a comparative study of the ionic composition and permeability of erythrocyte membranes in the capillary blood during the development of the inflammatory-dystrophic form of periodontosis.

EXPERIMENTAL METHOD

Erythrocytes were isolated from blood taken from the gums of a patient with periodontosis during curettage and also from blood taken from a finger. At the same time blood was taken from a finger for general analysis. Blood taken from the finger of clinically healthy persons served as the control.

The erythrocytes were separated, washed, and incubated in the same medium of the following composition: 0.3 M sucrose, 10 mM Tris-HCl, 50 units/ml heparin, pH 7.5. Blood in a volume of 0.1 ml was introduced into a centrifuge tube containing 1 ml of isolation medium and centrifuged for 10 min at 1000 rpm on the TsLK-1 centrifuge; the supernatant was drawn off and the residue was washed twice with 10 volumes of medium, after which the erythrocytes were sedimented by centrifugation.

The concentration of potassium ions passing into the incubation medium on the addition of erythrocytes was measured by means of a K^+ -selective membrane electrode [4]. In the experiments with valinomycin the K^+ concentration was 1 μ g/ml incubation medium. The coefficient

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TABLE 1. Results of Blood Analysis ($M \pm m$)

Group of subjects	Leukocytes	ESR
Control (healthy persons)	6059 \pm 279	10 \pm 1,0
Patients with periodontosis:		
I degree	6618 \pm 531	10,6 \pm 1,0
II-III degree	7978 \pm 828*	14,6 \pm 3,1

* $P < 0.02$ compared with control.TABLE 2. K^+ Content in Erythrocytes (C , g-ion/erythrocyte), Rate of K^+ Outflow from Erythrocytes (g-ion/min per erythrocyte), and Coefficient of Permeability (in cm/sec) ($M \pm m$)

Group of subjects	Blood	$C \cdot 10^{15}$	K^+ concentration in erythrocytes, mM	Outflow of K^+					
				immediately after isolation		after addition of valinomycin		1-2 h after isolation	
				$V \cdot 10^{17}$	$P \cdot 10^8$	$V \cdot 10^{17}$	$P \cdot 10^8$	$V \cdot 10^{17}$	$P \cdot 10^8$
Control (healthy persons)	From finger	7,2 \pm 0,3	80 \pm 3,3	7,9 \pm 1,1	12,7 \pm 1,3	27,7 \pm 3,3	72,2 \pm 5,6	12,5 \pm 0,6	20,2 \pm 0,1
Patients with periodontosis: II-III degree P	From finger	11,7 \pm 0,8 <0,001	130,0 \pm 8,9 <0,001	9,1 \pm 0,6	10,7 \pm 0,1	38,1 \pm 3,0 <0,02	93,4 \pm 4,8 <0,01	15,1 \pm 4,3	17,9 \pm 3,6
	From gums	13,2 \pm 0,7 <0,01	146,7 \pm 7,8 <0,001	11,6 \pm 2,3	10,2 \pm 1,4	52,1 \pm 8,2 <0,02	80,0 \pm 6,3	30,1 \pm 8,4 <0,05	26,3 \pm 5,7
I, II, III degree P									

of permeability of the erythrocyte membranes (P) was calculated by the equation: $V = P \cdot S(C_2 - C_1)$, where V is the velocity of K^+ transport, S the surface area of an erythrocyte; C_1 and C_2 the concentrations of potassium ions inside the erythrocyte and in the incubation medium, respectively. The number of erythrocytes added to the medium was determined by direct counting in a Goryaev's chamber.

EXPERIMENTAL RESULTS

Altogether 32 persons aged from 16 to 45 years were subjected to clinical and roentgenologic investigation: 12 were classed as clinically healthy (control group), periodontosis of the I degree was observed in 11, and periodontosis of the II-III degree in nine subjects. Blood was taken both from the gums and from the finger of the patients with periodontosis. Blood was taken from the finger of all 32 subjects for general analysis.

In 10 of the patients there were no concomitant diseases, five patients suffered from cardiovascular diseases, four from diseases of the gastrointestinal tract, one had chronic pancreatitis, and one had urolithiasis.

No concomitant diseases were found in five subjects in the control group, two had cardiovascular diseases, one had chronic tonsillitis, two had diseases of the gastrointestinal tract, one had chronic pancreatitis, and one had undergone hemiresection of a lung in the past for pleurisy.

Laboratory analysis of the blood showed an increase in the leukocyte count in patients with the II-III degree of periodontosis (Table 1). The results of analysis of the K^+ metabolism system in the erythrocytes of healthy subjects and patients with periodontosis are given in Table 2. With an increase in the severity of the disease the K^+ concentration in erythrocytes of blood taken both from the gums and from the finger was increased. For example, whereas in patients with established periodontosis of the I degree the K^+ content in erythrocytes from blood from the finger was increased to $7.8 \cdot 10^{-15}$ g-ion per erythrocyte, not significantly different from the control, in patients with degree II-III of periodontosis the content and concentration of K^+ in the erythrocytes differed highly significantly from the control group (Table 2). Even sharper changes in the K^+ content were observed in erythrocytes from blood taken from the patients' gums. In patients with periodontosis of the I degree the potassium content in erythrocytes from blood taken from the gums was virtually the

same as that in patients with degrees II and III, so that they could all be included in the same group. In the control group, just as in the group of patients with different degrees of periodontosis, the rate of outflow of K^+ from the erythrocytes, measured immediately after their isolation, was relatively small. There were cases when no potassium ions whatsoever left the erythrocytes. After standing in a concentrated suspension at room temperature for 1-2 h and subsequent addition to the incubation medium, in all cases considerable escape of potassium ions was recorded, especially from erythrocytes isolated from blood taken from the gums of patients with periodontosis.

Valinomycin increased the rate of outflow of K^+ both in the control group and in the patients, but to different degrees. A difference was observed even with freshly isolated erythrocytes. The rate of outflow was considerably higher in patients than subjects of the control group. The increase in the rate of outflow of potassium ions into potassium-free incubation medium from erythrocytes of patients with periodontosis compared with the control group can be explained by their higher potassium content. The membrane permeability under these circumstances could remain unchanged. To estimate the resistance of the erythrocyte membranes, the coefficient of permeability of potassium ions through the membrane was calculated.

The results showed no significant difference between the coefficients of permeability of the erythrocyte membranes of healthy subjects and patients with periodontosis for potassium ions. Consequently, the increased K^+ concentration in the erythrocytes found in these experiments can be explained by the more intensive active accumulation of intracellular K^+ .

Since the metabolism of erythrocytes is directly dependent on their ionic composition, the disturbance of the K^+ content in the erythrocytes observed in patients with periodontosis could affect their basic function of gas exchange. This would lead to the intensification of hypoxia in those areas of the tissues where the circulation is disturbed, as is the case in periodontosis.

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