Department of Physiology (Head: prof. dr. Gh. Arsenescu),
Institute of Medicine and Pharmacy, Tirgu Mureş

IN VITRO STUDIES OF PHOSPHORUS UPTAKE BY HUMAN NORMAL ERYTHROCYTES OF THE ABO BLOOD GROUPS

G. *Badiu*

Over 250 different antigenic groups ("determinants") have been described for the surface of the red blood cell (3). The great attention is now focused on the ways in which cell surfaces, with a lot of surface receptors, interact with other biological processes. In order to approach also further aspects of the behaviour of erythrocytes, we studied in vitro the phosphorus uptake by normal membrane erytrocytes of different blood groups (ABO system).

Material and method

In vitro determinations of the uptake of phosphorus by normal erythrocyte membranes were performed in a group including 25 adult healthy persons from each blood groups (0I, AII, BIII and ABIV). Sampling of 10 ml of blood from the cubital vein was done on an anticoagulant (heparin).

In order to study the uptake of phosphorus, labelled erythrocytes were used from a blood sample with radioactive phosphorus, according to the tehnique described by *Nicolau* (1959) in which we made a modification (1, 4). To this quantity of blood were added 0.10 ml of a solution of radioactive phosphate ($Na_2H^{32}PO_4$) supplied by the Institute of Atomic Physics (Bucharest), which contained 5 μCi. After the blood and the radioactive substance were thoroughly mixed, a 2-hour incubation at 37 °C was done. After incubation the blood was centrifuged for 5 min at 2000 rotations minute. The separated plasma was disposed of and the red cell sediment was treated with an equal volume of physiologic saline. After thorough mixing by means of a Pasteur pipette, centrifugation was done again in the same conditions as above, repeating the washing 4 times. A final suspension of ^{32}P-labelled erythrocytes was obtained. Adding to 2 ml of the erythrocyte suspension an equal volume of physiologic saline, a 50 % suspension of labelled erythrocytes was obtained. Of this suspension 0.5 ml were put in a flask, adding 125 ml of NaOH 0.02 N (dilution 1:500) and waiting 20 minutes for hemolysis to occur. After the production of hemolysis, radioactivity measurement was proceeded. 1 ml of the hemolyzed solution was measured in special targets and then vaporized in a bath for 1 hour, after which radioactivity was assessed with an RFT particle counter with solid (crystal) scintillator for beta radiations.

Results

The results (expressed in counts per minute) are shown in Table I.

Table I.

Blood group	Number of determinations	Values ± ESM (counts/min)	Significance
$^{0}_{I}$	25	59±3.2	—
$^{A}_{II}$	25	51±2.0	nonsignificant
$^{B}_{III}$	25	64.8±4.1	nonsignificant
$^{AB}_{IV}$	25	69.0±4.9	nonsignificant

The Student ("t") test applied in order to compare the arithmetic means of ^{32}P-uptake of the AII, BIII and ABIV erythrocytes group to the 0I group without antigens A, B demonstrated nonsignificant difference.

Discussion

Red blood cell membranes have probably been studied more than any other membrane, because they are semipermeable and serve as model to study permeability to the passage of polar ions and molecules. Many studies have been performed with the ^{32}P-labelled phosphorus, as the permeability rate of this ion is lower than that of other ions, and the phosphorus ion plays an essential role in the metabolism of the red cell (2).

Although many complexities remain, recent investigations have established the structure of antigenic determinants and have elucidated some biochemical differences for the ABO blood groups. It is now admitted that there are two types of terminal nonreducing ends in each molecule of blood antigenic groups, both types of chains terminate in α-linked N-acetylgalactosamine in A type group, but in galactose in B type group, while in 0 blood group this terminal residue is completely lacking and the chains are one unit shorter than in case of the A and B antigen, and AB group are heterozygous (3). All these carbohydrate residues of the proteic component of the red blood cell membrane (spectrine, glycoprotein or glycophorin) are very important by the fact that they carry blood group antigens and it has been suggested that these proteins regulate the penetration into cells of both ionic and nonionic substances (3, 5).

In spite of this antigenic and biochemical differences our data obtained show a nonsignificant differences between various erythrocytes of the ABO system, the behaviour of the red cell for the phosphours uptake is similar, and also that the uptake process is not influenced by the presence or absence of antigens of the ABO system.

References

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