PHYTOHEMAGGLUTININ EFFECT ON THE ELETROPHORETIC MOTILITY OF ERYTROCYTES

D. Kovachev, H. Divanyan

The electric charge of cells plays an important role in all physiological processes. To secure normal fulfilment of their functions, the blood cells should possess a stable electric charge which is conditioned by the chemical structure of the cellular membrane, and by the composition of the surrounding milieu. This charge is altered in pathological conditions and as the result of external effects (1, 6). In this regard, it is of utmost importance to determine the change in electrophoretic motility (EPM) of the cells caused by a variety of external factors, and in morbid conditions as well.

According to Roquet (5), there are phytohemagglutinin (PHA) receptor zones on the cellular surface whose number varies in the course of the subject's individual development. Lesney (4) and Stuart (7) point out that the quantity of these zones on the erythrocyte membrane is different for PHA obtained from various vegetable species, with the quantity for PHA from Phaseolus vilgaris amounting to 520 000, for Robinia pseudoaccacia — 210 000, and for Lens culinaris — 5.5×10^5.

PHA «Phaseolosaxin» is characterized by a high mitogenic and blast activity, and marked proliferative processes' stimulation (2). The preparation was obtained in the Chair of Biology at the Medical Faculty — Varna, after the method of M. Tzoneva (3). It is the purpose of the present work to investigate the action of PHA phaseolosaxin on EPM of erythrocytes, in vitro.

Material and methods

Rabbit erythrocytes, undergoing three-fold washing, were used in the study. The erythrocyte mass obtained wes diluted with physiological saline at 1:10 ratio. Rabbit red blood cells were used to render possible the comparative study of the results from planned future studies, in vivo. Under experimental conditions, the erythrocytes were diluted in physiological saline containing 20 γ/ml PHA. The indicated PHA concentration exerts maximum mitogenic effect on the blood cells, and it is the most frequently employed in cytogenic researches. Red blood cells, diluted with physiological saline were used as a control. To determine EPM of the erythrocytes, we measured the time required by the cell to move at a certrain distance within a microchamber, designed and constructed at the Chair of Biophysics, Biological Faculty — Sofia University. Measurements were carried out with ocular mesh and secundometer, observing sets of 50 cells each in microscope at one-fifth depth level of the chamber, at tension 180 volts and current force 4.5 and 6 milliamperes. The different force employed contributes to the reliability of the results. The time required by erythrocytes to cover 1 mm distance — from right to left from left to right and vice versa — was measured three times.

Results and discussion

Table 1 illustrates the results of the study performed.

Table 1

Pha effect on erythrocyte EPM

Group	Current force	Number of cells	x_{sec}	M	P
Exper.	4	50	8.77	8.77±0.34	<0.01
Control	4	50	5.70	5.70±0.19	
Exper.	5	50	6.80	6.80±0.42	<0.01
Control	5	50	4.09	4.09±0.11	
Exper.	6	50	3.78	3.78±0.12	>0.05
Control	6	50	3.80	3.80±0.10	

The data from each experiment were compared separately with the control. Reliability of the results was calculated according to the variational analysis rules.

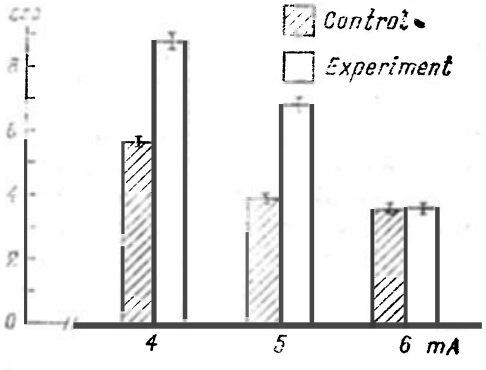

Fig. 1. Correlation between time for erythrocyte movement and electric current force.

In the control setting, at current force 4 mA, the mean time for displacement of erythrocytes at 1 mm distance is 5.70 sec, whilst in PHA treated erythrocytes — 8.77 sec, at $P<0.01$. At 5 mA, the time values are accordingly 4.09 seconds for controls, and 6.80 seconds for the experimental groups. Only when 6 mA are used, there is no essential difference between control and experimental groups. The above results are clearly demonstrated in Figure 1.

The data obtained show that at force of the current 4 and 5 mA, the time required for cell movement increases as the result of electric potential fall, whereas at 6 mA, the diference in velocity of movement of the erythrocytes between control and experimental groups disappears. The observed reduction of the electric charge may be attributed to the fixation of PHA molecules on the surface of the cells. The method applied did not allow for detailed elucidation of the mechanism of this particular phenomenon. It is furthermore evident that changes in the potential under the influence of PHA might de recorded only upon application of a suitable electric field.

The results presented clarify a single aspect of the biological action of PHA, and they should be beared in mind during its application, both in vivo, and in vitro.

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ВЛИЯНИЕ ФИТОГЕМОГЛОБУЛИНА НА ЕЛЕКТРОФОРЕТИЧЕСКУЮ ПОДВИЖНОСТЬ ЭРИТРОЦИТОВ

Д. Ковачев, Х. Дизанян

РЕЗЮМЕ

Определена электрофоретическая подвижность эритроцитов зайца в микрокамере. Прослежено время перемещения на определеное расстояние. Наблюдается снижение электрического заряда красных кровяных клеток под действием 20γ/мл ФХА. Допускается, что ФХА фиксируясь на клеточной мембране ведет к изменению электрического заряда. Эти изменения наблюдались только в соответствующем электрическом поле.