# EFFECT OF $Ca^{++}$ AND $Mg^{++}$ IONS ON THE PLATELET ELECTROKINETIC POTENTIAL IN ONTOGENESIS

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In rabbits of different age groups $Ca^{++}$ ions increase while $Mg^{++}$ ions decrease the platelet electrokinetic potential.

Platelets carry a negative charge on their surface [1]. Since the platelet circulates in an electrolyte medium, an electrical double layer consisting of ions of opposite sign is formed at the boundary separating the two phases. The adsorption layer on the cell surface determines the magnitude of its surface charge, while the diffuse layer lies in the surrounding medium. The electrokinetic potential of the cell depends on the structure of and interaction between these layers. The formation of the double electrical layer around the platelet can be affected not only by the properties of the outer membrane on the platelet itself, but also by changes in the ionic composition of the surrounding medium and, in particular, by an increase in the concentration of bivalent cations.

Since the possibility cannot be ruled out that in the various stages of ontogenesis the platelet membrane may possess different adsorption and ionogenic properties, it was decided to investigate whether changes take place in the electrokinetic potential of platelets under the influence of $Ca^{++}$ and $Mg^{++}$ ions at different stages of ontogenesis.

## EXPERIMENTAL METHOD

The Opton Cytoferometer was used in the investigation. The electrophoretic mobility of the cells was measured in the anterior stationary zone of the flat vertical chamber, 14 mm high and 0.7 mm deep. The temperature was controlled at 25 ± 0.02°C by means of a Colora Type-K ultrathermostat. The cells moved in a steady electric field with a current strength of 5 mA. Their movement was observed in the phase-contrast microscope under a magnification of 800 times. The medium for measurement of mobility was Michaelis buffer, pH 7.35. The electrophoretic mobility of the platelets was calculated from the formula

$$B = \frac{S}{n \cdot E},$$

where S is the path of the platelet in the grid of the ocular micrometer (in cm); n the time taken by the platelet to travel along this path (in sec); E the intensity of the electric field. This was calculated from the formula

$$E = \frac{I \cdot p}{h \cdot t},$$

where I is the strength of the current (in A); p a special constant of specific resistance of the Michaelis buffer, namely 68.64 Ω/cm; h the height of the chamber (in cm); and t the depth of the chamber (in cm).

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TABLE 1. Effect of $Ca^{++}$ and $Mg^{++}$ Ions on Electrophoretic Mobility of Platelets in Vitro

| Age groups of rabbits | Initial electrophoretic mobility of platelets, in $cm^2/V \cdot sec$ | Electrophoretic mobility after incubation of 1 ml plasma with 0.2 ml 0.1 M $CaCl_2$, in $cm^2/V \cdot sec$ | Electrophoretic mobility after incubation of 1 ml plasma with 0.2 ml 0.1 M $MgCl_2$, in $cm^2/V \cdot sec$ |
|---|---|---|---|
| 9 days | $0{,}907 \cdot 10^{-4} \pm 0{,}017 \cdot 10^{-4}$ | $0{,}982 \cdot 10^{-4} \pm 0{,}020 \cdot 10^{-4}$ $P<0{,}001$ | $0{,}786 \cdot 10^{-4} \pm 0{,}031 \cdot 10^{-4}$ $P<0{,}001$ |
| 15 days | $0{,}731 \cdot 10^{-4} \pm 0{,}009 \cdot 10^{-4}$ | $0{,}922 \cdot 10^{-4} \pm 0{,}043 \cdot 10^{-4}$ $P<0{,}001$ | $0{,}672 \cdot 10^{-4} \pm 0{,}027 \cdot 10^{-4}$ $P<0{,}001$ |
| Adult | $0{,}784 \cdot 10^{-4} \pm 0{,}013 \cdot 10^{-4}$ | $0{,}836 \cdot 10^{-4} \pm 0{,}012 \cdot 10^{-4}$ $P<0{,}002$ | $0{,}723 \cdot 10^{-4} \pm 0{,}016 \cdot 10^{-4}$ $P<0{,}005$ |

TABLE 2. Effect of $Ca^{++}$ and $Mg^{++}$ Ions on Electrophoretic Mobility of Platelets in Vivo

| Initial electrophoretic mobility of platelets (in $cm^2/V \cdot sec$) | Electrophoretic mobility of platelets after injection of 10 ml 1% solution of | |
|---|---|---|
| | $CaCl_2$, $cm^2/V \cdot sec$ | $MgCl_2$, $cm^2/V \cdot sec$ |
| $0.794 \cdot 10^{-4} \pm 0.011 \cdot 10^{-4}$ P | $0.870 \cdot 10^{-4} \pm 0.017 \cdot 10^{-4}$ $<0.001$ | $0.731 \cdot 10^{-4} \pm 0.013 \cdot 10^{-4}$ $<0.001$ |

The electrokinetic potential of the cell was calculated by the formula

$$Z = \frac{4\pi\eta\beta}{D},$$

where $\eta$ is the viscosity of the medium surrounding the cell; D the dielectric constant of the medium; and $\beta$ the electrophoretic mobility of the cell per unit intensity of the electric field [2]. In these investigations the viscosity of the medium and its dielectric properties remained constant; only the electrophoretic mobility of the platelets reflecting changes in their Z-potential, was therefore determined.

Blood was taken by cardiac puncture and stabilized with 3.8% sodium citrate in the ratio 4:1. Platelet-enriched plasma was prepared from blood by centrifugation at 2000 rpm for 5 min.

Changes in the ionic composition of the plasma in vitro were induced by the addition of isotonic (0.1 M) solutions of calcium ($CaCl_2$) and magnesium salts ($MgCl_2$) followed by incubation at room temperature for 30 min. In a series of 10 experiments in vivo, adult rabbits were used; after determination of the original electrophoretic mobility of the platelets, 10 ml 1% $CaCl_2$ or $MgCl_2$ solution was injected intravenously, after which further blood samples were taken.

Altogether 112 young rabbits aged 9 days, 70 aged 15 days, and 56 adult rabbits were studied in the experiments.

In each determination the electrophoretic mobility of 30 different platelets was recorded.

## EXPERIMENTAL RESULTS

As the results given in Table 1 show, the platelet electrokinetic potential of rabbits aged 9 days is higher than that of rabbits aged 15 days and adult rabbits ($P < 0.001$).

Determination of the electrophoretic mobility of platelets from rabbits of the same age groups after incubation with isotonic $CaCl_2$ and $MgCl_2$ solutions showed that $Ca^{++}$ ions increase while $Mg^{++}$ ions decrease mobility.

Under the influence of $Ca^{++}$ and $Mg^{++}$ ions, aggregation of the platelets frequently occurred.

After intravenous injection of $CaCl_2$ solution into adult rabbits the electrophoretic mobility of their platelets increased appreciably, while it was reduced after injection of $MgCl_2$ solution (Table 2).

An increase in the $Ca^{++}$ ion concentration thus increases the electrophoretic mobility of platelets and, consequently, increases their electrokinetic potential. An increase in the $Mg^{++}$ ion concentration reduces the electrophoretic mobility of the platelets and reduces their electrokinetic potential.

## LITERATURE CITED


1. H. A. Abramson, J. Exp. Med., 47, 677 (1928).
2. Von Smoluchowski, in: Handbuch der Electrizität und Magnetismus, Leipzig (1921).