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AUTHORS: Furedi AA, Ohad I:

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BBA 4257

EFFECTS OF HIGH-FREQUENCY ELECTRIC FIELDS ON THE LIVING CELL

I. BEHAVIOUR OF HUMAN ERYTHROCYTES IN HIGH-FREQUENCY ELECTRIC FIELDS AND ITS RELATION TO THEIR AGE

A. A. FÜREDI AND I. OHAD

Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem (Israel)

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SUMMARY

Behaviour of human erythrocytes in high-frequency electric fields is described. When the field is applied erythrocytes show a reversible elongation which is accompanied by a rotatory motion. Old erythrocytes do not behave in this manner but form chains oriented in the direction of the field. The results can be explained by the interaction of two effects: (1) the polarization of the erythrocyte and (2) the distortion of the applied field by the fixed structural charges of the erythrocyte.

The method of separating young from old erythrocytes according to their specific gravity has been improved.

INTRODUCTION

Previous investigations have shown that microscopic particles become arranged parallel to the direction of electromagnetic fields in the radio-frequency range[1,2]. It was demonstrated that this phenomenon is the result of the induced dipole moment of the particle[3].

In the present work the behaviour of human erythrocytes in the high-frequency field has been investigated using the apparatus described before[3].

MATERIALS AND METHODS

Erythrocytes were obtained from heparinised fresh blood. After centrifugation at 300 × *g* for 5 min the cells were resuspended in aqueous sucrose solution (11%, w/v, for isotonic and 5%, w/v, for hypotonic solutions) and stored for the duration of the experiment (1–3 h) at room temperature.

Specimens for examination were prepared by applying a drop of the cell suspension on a microscope cover glass (22 × 22 mm) which was then covered by a second round cover glass (19 mm diameter) and placed on the electrodes under the phase microscope. Specimens were usually allowed to stand 1–2 min before the field was applied for 1–5 sec from a balance oscillator of 120 megacycles/sec. Longer exposure (> 10 sec) causes damage to cells by heating.

A simple and quick procedure developed for separating between young and old erythrocytes, was based on the method of PRANKERD[4]. Erythrocytes were sedimented as described above and the plasma was discarded. One volume (0.2 ml) of packed cells was mixed gently with two volumes (0.4 ml) of 30% (w/v) crystalline bovine albumin (Armour Pharmaceutical Co. Ltd.) in a 5 × 50 mm plastic tube (Spinco Co.). The mixture was centrifuged in the swinging-bucket rotor of the Clay-Adams serological centrifuge at 3000 rev./min for 30 min. Glass serological centrifuge tubes were used as adaptors for the small plastic tubes.

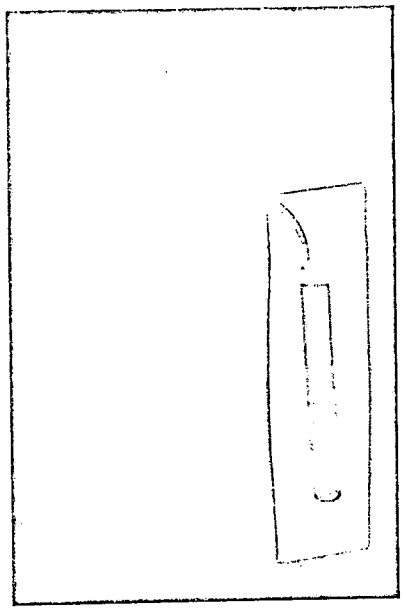


Fig. 1. Tube with separated erythrocytes. Top layer, young erythrocytes; middle layer, albumin solution; bottom, old erythrocytes.

Centrifugation causes the cells to concentrate into two distinct layers, with clear and sharp boundaries (Fig. 1), a top layer of about 12 mm and a bottom one of 2–3 mm, separated by about 20 mm of albumin. The top layer was found to consist of young cells and the bottom layer of old cells when examined by the methods and criteria of DANON AND MARIKOVSKY[10]. During the process of fractionation some hemoglobin is released to the albumin solution. To separate between layers the tube is cut into two in the middle of the albumin layer. The cells from each layer are washed in 2 ml isotonic sucrose and saline (1:1 by volume) and centrifuged as described above. The washed packed cells (0.08 ml) were mixed with 1 ml of sucrose (isotonic or hypotonic) and 0.04 ml saline for further examination in the electric field.

RESULTS

When the field is applied to erythrocytes suspended in isotonic sucrose solution they become oriented so that their radii are perpendicular to the direction of the field

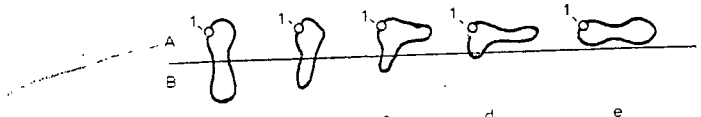


Fig. 2. Schematic drawing of the amoeba-like rotation of erythrocytes suspended in isotonic sucrose solution when an electric field is applied.

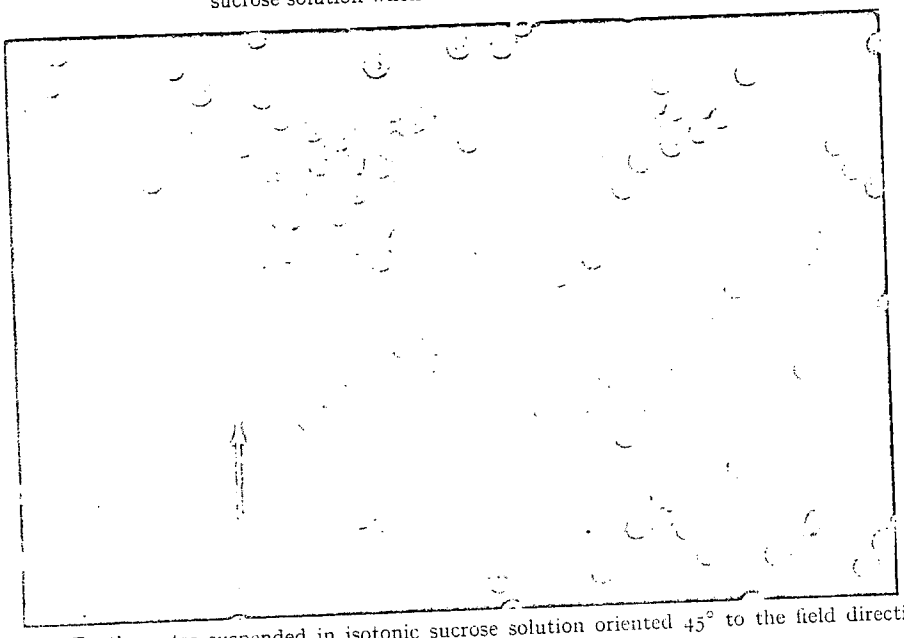


Fig. 3. Erythrocytes suspended in isotonic sucrose solution oriented 45° to the field direction (⟶).

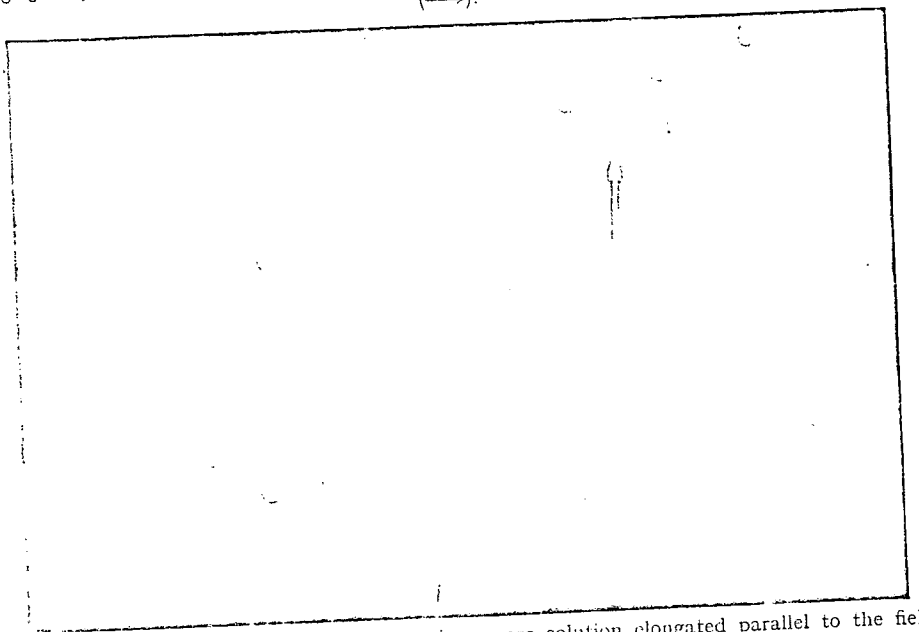


Fig. 4. Erythrocytes suspended in hypotonic sucrose solution elongated parallel to the field direction (⟶).

and rotate slowly. The rotation of the cells results from distortions of shape reminiscent of amoeboid movement (Fig. 2). The cell undergoes this change of shape in such a way that Point 1 (Fig. 2a) remains static while the upper part of the cell (A) increases in volume on account of the lower part (B). When the field is turned off while the shape of the erythrocyte is still distorted (Fig. 2b, c or d) it returns to its natural form (Fig. 2a or 2e). If the field is cut off when the cell has reached position e (Fig. 2) no further change in shape takes place, indicating that the cell has reached a stable form.

In addition to rotation, during a prolonged exposure to the electric field (5 sec) cells form long chains oriented about 45° to the direction of the field (Fig. 3).

Cells suspended in hypotonic sucrose solution lose their bidiscoidal form and swell to spherical shape. When electric field is applied they elongate so that their long axis is parallel to the direction of the field (Fig. 4) and rotate without changing its direction (Fig. 5). Elongation of erythrocyte is 2–4 times greater than the normal

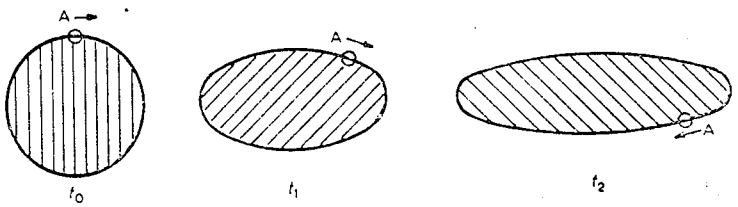


Fig. 5. Schematic representation of the effect of field on young erythrocytes in hypotonic medium. t_0 represents undistorted erythrocyte at zero time. t_1, t_2 show distortion and rotation of erythrocyte. Point A describes an ellipse around central axis. Cross hatching represents the orientation of the cell contents.

cell diameter. Addition of salt ($\geqslant$ 0.02% NaCl) stops cell rotation but does not prevent elongation. If the elongation is maintained for 5–15 sec, cells break up into small spherical vesicles which sometimes remain interconnected by long thin filaments. The process of vesicle formation seems to be preceded by a contact between opposite regions of cell membranes at points of maximum elongation. The opacity of the vesicles appears to be similar to that of whole cells indicating that there is no extensive leakage of the cell contents. Morphologically, they resemble the myelinic forms described by BESSIS[11].

Finally, prolonged application of the electric field results in excessive heat formation, cells break up and release their contents. The remaining ghosts are no longer affected by the field. These ghosts are granulated and differ in appearance from those obtained by gradual hemolysis[6] and which *do react* to the electric field similarly to intact cells.

Not all the cells in blood samples obtained from healthy donors became elongated under hypotonic conditions. This difference in behaviour may be explained by differences in membrane properties of the individual cells. Separating the cells into top (young) and bottom (old) fractions using the procedure described, it was found that more than 90% of the top fraction but less than 10% of the bottom fraction exhibited rotation and elongation (Fig. 6a, b). Old erythrocytes often form chains oriented in the direction of the field.

Fig. 6. Influence of field

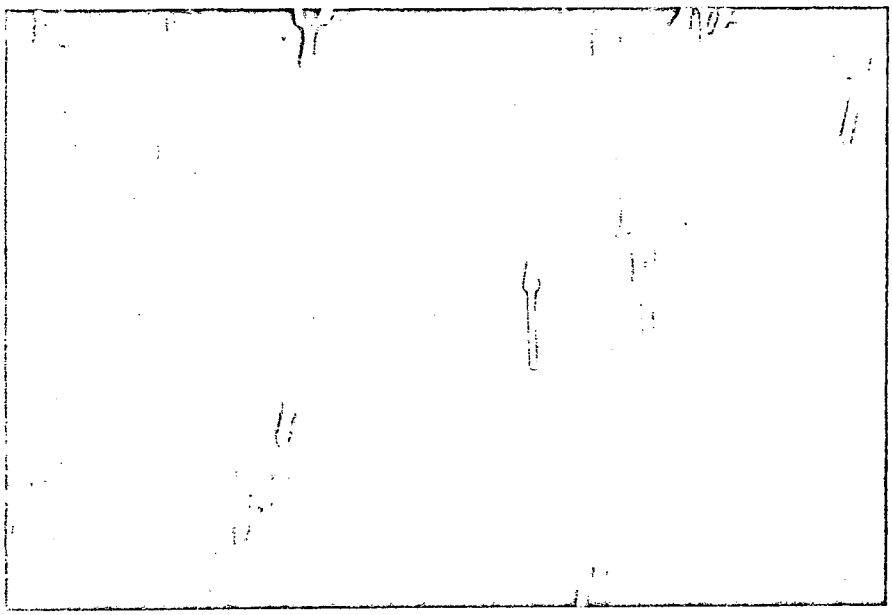


Fig. 6a.

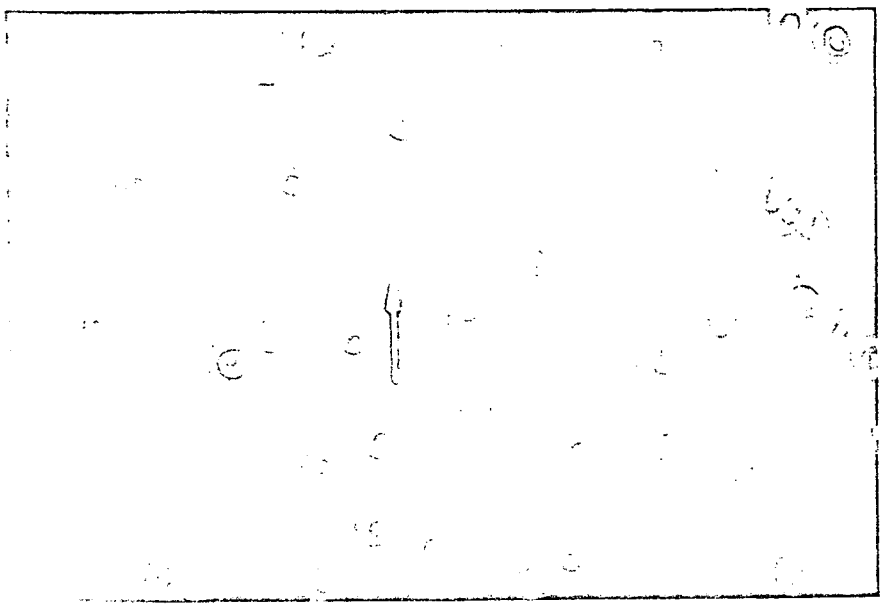


Fig. 6b.

Influence of field on erythrocytes separated into old and young fractions. a, top fraction (young); b, bottom fraction (old).

DISCUSSION

The various types of behaviour of erythrocytes in the high-frequency electric field can be explained in terms of their dielectric constant, structural charges, shape, elasticity and composition of the external medium.

The described behaviour of erythrocytes can be better understood if we first consider model systems: uncharged particles with a dielectric constant different from that of the surrounding medium which are polarized in a high-frequency electromagnetic field and form long chains parallel to the direction of the field[3]. If these particles were elongated they would align themselves with their long axis parallel to the lines of force (Fig. 7a). On the other hand, elongated particles with fixed structural charges align themselves at right angles to the applied field[7,8]. As a result of the distortion of the field, elongated particles would tend to assume a resting position perpendicular to lines of force where the field distortion and hence the potential energy is minimal (Fig. 7b). The degree of field distortion resulting from structural charges on the particle and the intensity of its polarisation mutually determine the final orientation. This prediction has been verified experimentally with bacteria which are charged elongated particles (B. HERSKOVITS, unpublished experiments). Raising the salt concentration or lowering the pH of the medium surrounding the bacteria causes them to reorient in the direction of the field. This is readily explained since the fixed charges are neutralised by added ions while the induced dipole is not affected[3].

On the basis of the above considerations, an attempt has been made to predict the behaviour of idealised particles in a high-frequency electric field. The influence of the field on rigid and elastic charged and uncharged spheres and ellipsoids is described in Table I.

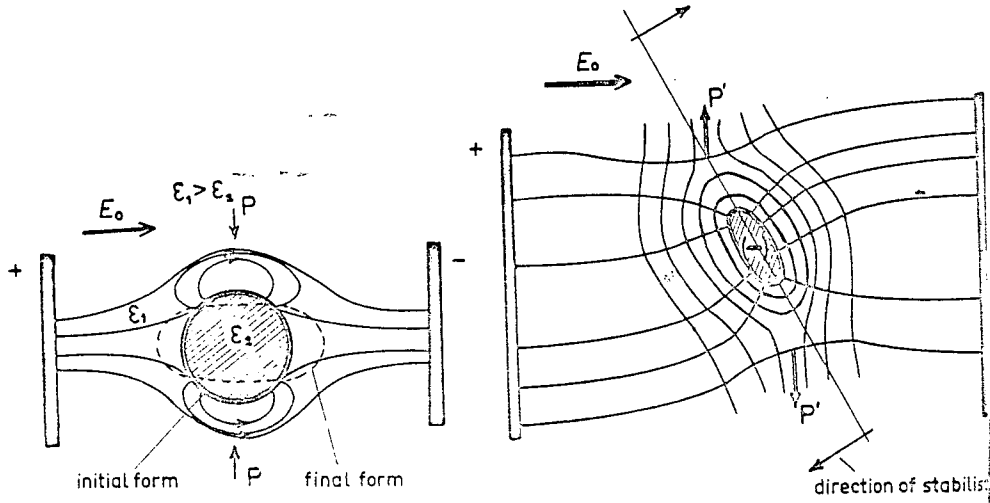


Fig. 7a. Fig. 7b.

Fig. 7. Schematic drawing of distortion of electric field by particles and the resulting stresses. Stress (P) resulting from field distortion according to MAXWELL[7] causes elongation. a. Elastic uncharged particle of low dielectric constant Stress (P) causes elongation and orientation parallel to the field (⟶) b. Rigid charged particle. Stress (P′) causes orientation perpendicular to field direction (⟶).

The elongation and rotation of young erythrocytes under hypotonic conditions (Fig. 5) corresponds closely to the behaviour predicted for elastic charged spheres (Table I). This could be explained if it is assumed that these cells are charged elastic discs with a dielectric constant lower than that of the medium. Apparently there are two conflicting influences on the erythrocyte: polarisation would tend to elongate them in the direction of the field, but as they bear fixed structural charges they would tend to align themselves perpendicular to the field, like charged elongated particles. This last alignment is not possible since the elongation which results from polarisation must be in the field direction. The overall result is that the particles rotate in the manner shown in Fig. 5.

Young erythrocytes in isotonic solution do not elongate and rotate more slowly in a characteristic manner (Fig. 2). This behaviour is too complicated to explain in terms of the idealized systems (Table I) but may be partly due to a higher charge density on the cell surface. The difference in behaviour between erythrocytes in isotonic and hyptonic solutions could be due to a decrease in charge density in the latter, resulting from swelling.

The addition of salt to young erythrocytes reduces the effect of their charge, thus inhibits rotation and they exhibit the behaviour of uncharged elastic spheres (Table I).

Old erythrocytes in both hypotonic and isotonic solution do not elongate or rotate, but can form chains oriented parallel to the field direction like rigid uncharged spheres (Table I). A possible explanation of the difference in behaviour between old and young cells is that decreasing lipid content during aging[4] increases their dielectric constant while their charge density decreases[9] and hence they become less sensitive to the field. Differentiation between young and old erythrocytes has been based on chemical properties, radioactive tagging[4] and on morphological studies[5]. In the present work two other possible methods for the quantitation of old and young cells in blood samples are described. Firstly, the modification of the method of PRANKERD combined

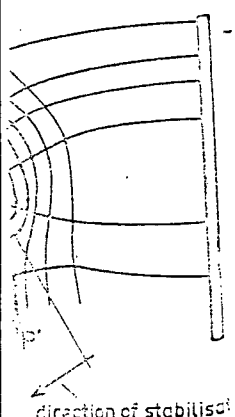
TABLE I

THEORETICAL BEHAVIOUR OF SPHERES AND ELLIPSOIDS OF DIELECTRIC CONSTANT DIFFERENT FROM THAT OF THE MEDIUM IN A HIGH-FREQUENCY ELECTRIC FIELD

The addition of salt transforms charged particle to uncharged.

			Change in shape	*Rotary motion*	*Orientation in the field direction*	*Orientation perpendicular to the field*	*Chain formation*	*Roulaux*
Spheres	Rigid	Charged	−	−	−	−	−	+
		Uncharged	−	−	+*	−	+	−
	Elastic	Charged	+	+	−	+	−	−
		Uncharged	+	−	+*	−	+	−
Ellipsoids	Rigid	Charged	−	−	−	+	−	+
		Uncharged	−	−	+*	−	+	−
	Elastic	Charged	+	−	−	+	−	−
		Uncharged	+	−	+*	−	+	−

* Chain-type orientation.



resulting stresses.
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with the estimation of haemoglobin in the two fractions, could be conveniently used clinically. Secondly, the difference in behaviour of young and old cells in the electric fields combined with photomicrography could be applied to differential counting of the two types of cells (elongated *vs.* round).

The possibility exists that under more carefully controlled conditions, examination of erythrocytes in high-frequency electric fields could be applied to the study and detection of pathological conditions.

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