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ROTATION OF ERYTHROCYTES, PLANT CELLS, AND PROTOPLASTS IN AN OUTSIDE ROTATING ELECTRIC FIELD

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According to the method published by ARNOLD and ZIMMERMANN 1982 human erythrocytes, suspension cultured cells of Beta vulgaris, protoplasts and isolated vacuoles were investigated. The cells rotate in the rotating electric field of a four electrode system. The rotation, defined as the torque per square field strength seems to be independent of the cellular diameter. The frequency for maximal rotation allows us to calculate specific membrane capacity or an effective capacity of plant cells sourrounded by cell wall, resp. This method also provides a sensitive measure of changes in membrane resistance since ionophores incorporated into the membrane innibit the torque in a reversible manner.

Introduction

The measurement of electric parameters of cells in suspension allows the determination of physical membrane properties such as membrane capacity and resistance. On the basis of such investigations, the specific capacity of the cell membrane was determined as 1 $\mu F/cm^2$. (A survey on the large number of these papers, allready started by K.S. COLE in 1938 has recently been given by ZIMMERMANN /20/). The disadvantage of this method, however, consists in the fact that only mean values of a large number of cells can be obtained in this way. Even relatively homogeneous cell populations, such as e.g. erythrocyte suspensions indicate differences in biophysical properties of individual cells.

As ARNOLD and ZIMMERMANN /1,2/ have shown recently, single cells spin in a rotating electric field in the case of resonance of the induced dipole. For this a four electrode chamber was developed, where the electrodes are driven with sinusoidal voltages having progressive 90° phase differences. Therefore at least in the center of this device an homogeneous field was cre-

ated, the vector of which rotates. The cells rotate in this field against the direction of the field vector and at a much slower frequency. From the field frequency which gives optimal cellular torque, the membrane capacity can be calculated. In contrast to impedance measurements in suspensions, only a small number of cells are necessary for this determination and in some cases even measurements of single cells are possible.
In this paper we present data on human erythrocytes and plant cells obtained by this method.

Methods

Preparation of cells: Human red blood cells stored no longer than two days in ACD medium were used. In some experiments the cells where washed in isotonic phosphate buffered NaCl solution, in most cases, they where diluted directly in low ionic strength sucrose solution to a hematocrite of 0.1%. The isotonic sucrose solution contained 1 mM phosphate buffer and the ionic strength was increased by additional 5 ...15 mM NaCl. For solutions with extremely low conductivity, only 0.1 or 0.5 mM phosphate were used (pH 6.7).
The Avena sativa mesophyll protoplasts were prepared as described by ZIMMERMANN and SCHREURICH /21/ and the vacuoles were obtained by the mechanical lysis of the protoplasts. For other experiments suspension-cultured cells and cell aggregates of Beta vulgaris were used. Cells were cultivated for 14 days in medium M3 modified after /11/. All cells were in the stationary phase.
Measuring setup: The rotation of cells was measured in a 4-electrode system which employed sinusoidal voltages with variable amplitudes in the frequency range of 100 Hz to 3 MHz in four phases, spaced by 90° and controlled by an oscilloscope. The electrodes (platinum or stainless steel) formed the sides of the square chamber fixed on a microscopic cover slip. The electrode distance varied between 2 and 6 mm. In case of plant cells the microscopic observation was made by a normal objective (8*), but for red cells a special long distance mirror objective (VEB Carl-Zeiss, Jena) 40* was used.
Plant cells were held between two layers of solutions of different densities (protoplasts and vacuoles between 0.5 M sucrose and 0.5 M mannitol, suspension cultured cells between 0.5 M suc-

rose or 0.2 M metrizamid and 0.1 ... 0.2 M mannitol). In these cases measurements of single cells at various frequencies are possible. Erythrocytes were measured during free sedimentation. Therefore every point of the curves in Fig. 2 represents 6 ... 15 single values of different cells (the bars indicate the standard deviation of these values, the marked segment the deviation of the mean value).

Results and discussion

Typical results of single experiments are demonstrated in Figs. 1 and 2. In the case of Beta vulgaris cells (Fig. 1) all points are from single measurements and each curve, corresponding to a definite ionic strength solution has been derived from a single cell. The points were fitted by the least square method, using the following function, derived from theoretical considerations/7,1/:

$$R = \frac{\omega}{E^2} = R_{max} \frac{2\,k\,f}{1 + (k\,f)^2}$$

Where k is the time constant (in s), f the frequency of the applied alternating field (in Hz) and ω the speed of rotation in rad/s. From the theoretical point of view the value N should be proportional to the applied field (E) and the dipole moment. This dipole itself is induced by the field, and its moment therefore is proportional to the field strength. Therefore N should be proportinal the square of the field strength. As indicated in Fig. 3 such a quadratic proportionality was found to good approximation. It seems reasonable to use the parameter "rotation" (R) as defined in the formula above since it is analogous to "mobility" in cell electrophoresis (electrophoretic velocity per applied field strength).

In the case of maximal resonance, k*f=1, the rotation reaches the maximum R_{max}. The calculated curves indicate a relative regression of 70 ...98 % for plant cells. The deviation of human red blood cells was larger (sometimes about 50%) because of the variability of the cells. Comparison of Figs. 1 and 2 indicates that the rotation did not depend strongly on the diameter of the cells. Nearly the same values were obtained for red cells (diameter 6 um) and cells of Beta vulgaris (diameter 125 µm). The reason for this seems to be that the dipole indiction depends on

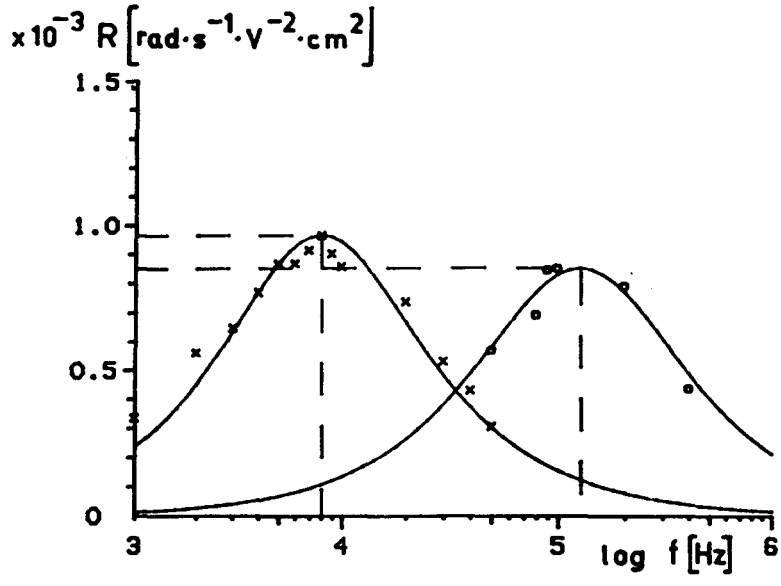

FIG. 1
Rotation (R) of single plant cells (Beta vulgaris) suspended in two different solutions (x---x = 13.5 µS/cm, o---o = 545 µS/cm) as a function of frequency (f).(External medium: 0.2 M mannitol, diameter of the cell = 125 um(x),and 108 µm(o) resp.)

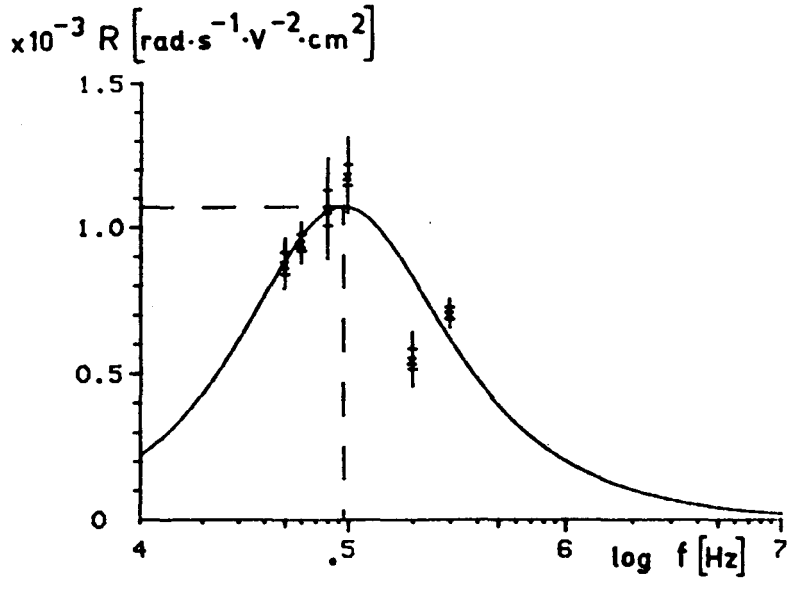

FIG. 2
Rotation (R) of human erythrocytes as a function of frequency (f). (External medium: 0.5 mM phosphate in 300 mM sucrose, G_e= 55 µS/cm.)

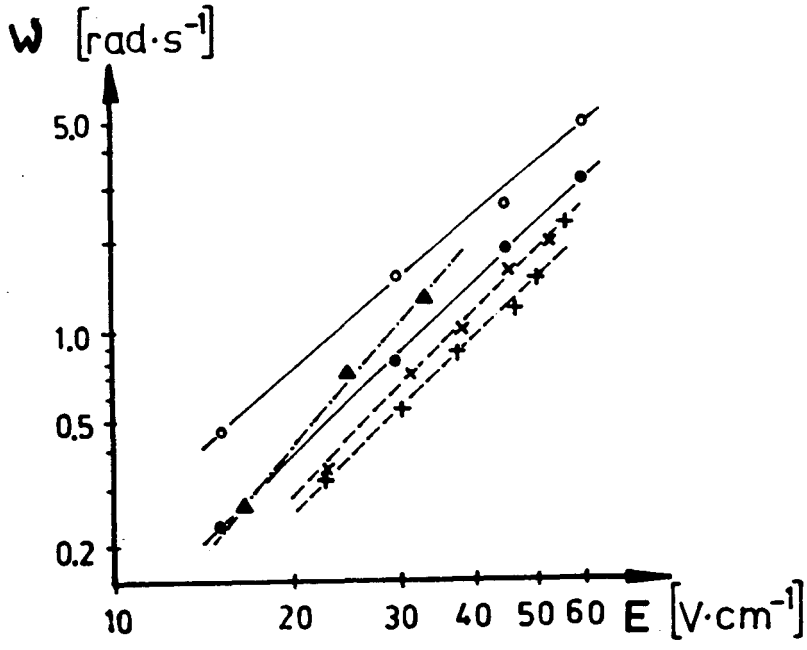

FIG. 3
Dependence of the rotation speed (ω) on applied field strength (E). (Parameters and symbols see Tab. 1)

TAB. 1
Parameters of the cells listed in Fig. 3

SYMBOL	CELL-TYP	EXT. CONDUCT. [µS/cm]	DIAMETER [µm]	FREQUENCY [kHz]
●---●	Avena sat. protoplast	85	37	50
o---o	Avena sat. vacuole	85	62	50
▲---▲	hum. erythrocytes	55	6	100
+---+	Beta vulg. single cells	27.5	120	30
x---x	-"- 3-cell aggregates	27.5	150	30

the diameter in the same way as the frictional force for rotation on spheres /7/. Therefore the dipole moment, increasing by the third power of the diameter was compensated by the frictional force. On the other hand a decrease of R_{max} in solutions of increasing ionic strength could be detected (Fig. 4). This dependency obviously results from the diminishing difference between the conductivities of electrolyte outside and inside the cells. The condition sine qua non for rotation is the high electric resistance of the membrane. Destroyed cells of Beta vulgaris or

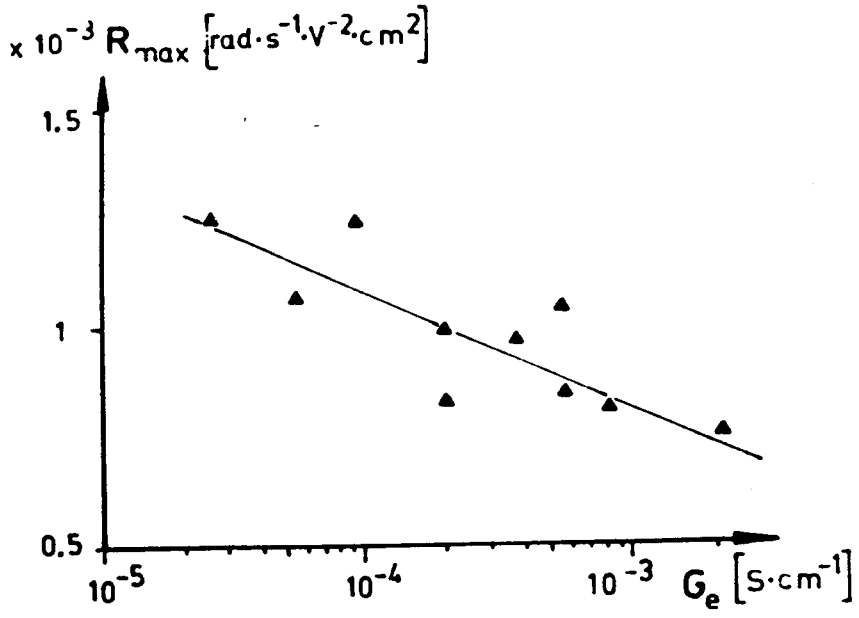

FIG. 4
Maximal rotation (R_{max}) of human erythrocytes in isotonic solutions as a function of the conductivity (G_e) of the outside medium.

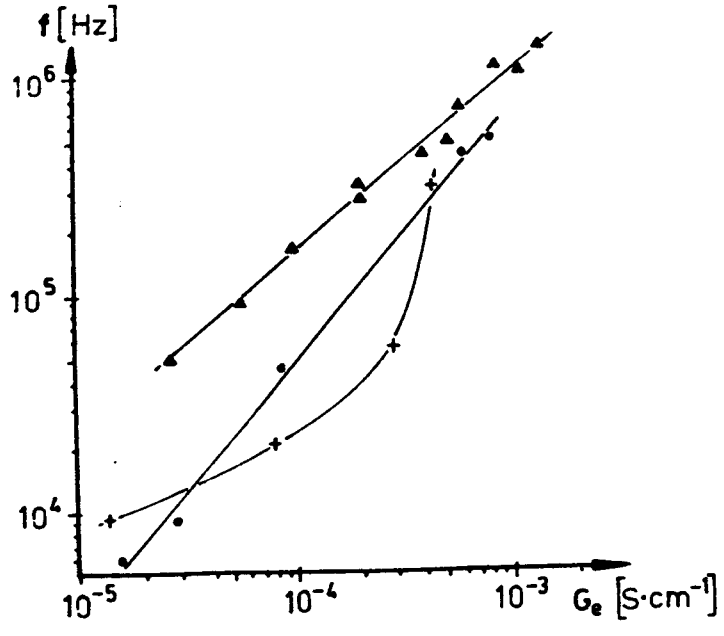

FIG. 5
Dependence of characteristic frequency (f) on external conductivity (G_e) ▲ = human erythrocytes, ● = Avena sativa protoplasts, + = Beta vulgaris single cells.

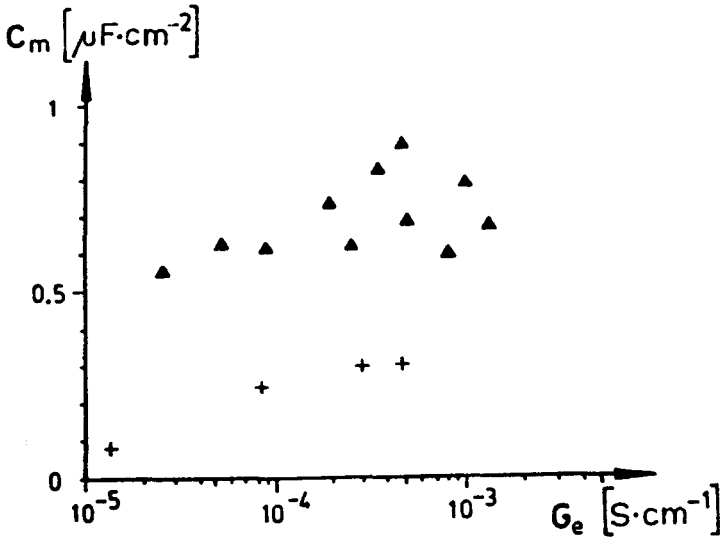

FIG. 6
Dependence of specific membrane capacity (c_m) of human erythrocytes (▲) and single cells of Beta vulgaris (+) on external conductivity (G_e)

cells treated with methanol, toluol or denaturated by heating do not show any rotation effect. Addition of an ionophore (2.5 µg/ml nystatin) to the suspension of erythrocytes completely stopped the rotation within 5 minutes. On the other hand, cells treated with nystatin in isotonic 30 mM NaCl-sucrose solution for one hour and subsequently washed in nystatin-free solution indicate a 3 times lower, but clearly measurable rotation at nearly the same resonancy frequency as normal cells. This indicates that the rotation seems to be a useful test for the membrane integrity of individual cells and thus we are able to confirm our assumptions made in previous experiments on shape changes /6/.

As indicated in Fig. 5, the frequency of maximal rotation for all the measured cells increases as the conductivity of the outside medium increases. The maximum conductivity used in this experiments was in the range of 1 mS/cm, corresponding to about 15 mM NaCl-solution. In solutions of higher ionic strength, turbulence near the electrodes occurs and an increase in temperature during measurement. On the other hand in lower ionic strength media, this effect is neglible. In solutions with a conductivity of 0.1 mS/cm e.g. by an applied field strength of 25 V/cm, a current density of 2.5 mA/cm^2 occurs. This leads to maximum temperature increase of 0.015 K/s if we consider that the ohmic heat is

quantitatively conserved in the suspension.
According to the theoretical consideration for the behaviour of isolated spheres, suspended in a medium with the conductivity G_e and indicating an inside conductivity G_i, the specific membrane capacity (c_m) can be calculated as a function of the frequency of maximal torque/1,7/:

$$c_m = \frac{1}{2 \pi r f (1/G_i + 1/2G_e)}$$

The measuring device did not allow an exact measurement of the erythrocyte diameter. The cells are slightly shrunken and show various forms. If the radius is taken as 3 µm and G = 5.2 mS/cm /12,13,14,18/ the specific capacities could be obtained as indicated in Fig. 6. Usually for cellular membranes specific capacities are assumed near 1 $\mu F/cm^2$ /1,5,13,18,20/. The difference in our results could be caused by using a wrong radius or by the hydrodynamic effects of nonspherical cells. As Fig. 6 indicates, we cannot be sure that the specific capacity of erythrocytes is independent of the ionic strength of the medium applied. The values in solutions of higher outside conductivities obviously depend strongly on the inner conductivity.
In the case of plant cells, the radius could be determined exactly. Measurements of cell homogenates indicate an inner conductivity of 4.5 mS/cm . For those conditions the specific capacity of the protoplasts was determined between 0.24 ... 0.40 $\mu F/cm^2$. This is near the value determined by ARNOLD and ZIMMERMANN /1/ and caused obviously by the fact that in this case a membrane system consisting of tonoplast and plasmalemma is present.
In the case of complete plant cells surrounded by the cell wall, the electrostatic model looks much more complicated. As clearly indicated in Fig. 5 the values of Beta vulgaris cells could not be connected by a linear logarithmic plot like the values for erythrocytes and protoplasts. Therefore the capacities indicate a clear ionic strength dependence (Fig. 6). In this case, the cell wall apparently behaves like an ion-exchanger whose dielectric properties strongly depend on the outside electrolyte /3, 4/. It is possible that even in erythrocytes and other cells the electrostatic charges in the surface coat have a similar effect /19/. These experiments indicate that the measurement of cell

rotation in a rotating field gives important information about membrane capacitance and resistance. In contrast to the measurement of passive electric properties of cell suspensions far fewer cells are necessary and individual properties of different cells in the same suspension can be ascertained. In this respect further quantitative data could be obtained as in dielectrophoretic measurements /8, 10, 17/. Previous measurements of the rotation properties of cells have made it possible to determine the optimal parameters for dielectrophoretic cell separation, as described by various authors /8, 9, 15, 16/.

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На основе метода опубликованного АРНОЛДОМ и ЦИММЕРМАННОМ в 1982, эритроциты человека, клетки сахарной свеклы, культивированных в суспенсиях, протопласты и вакуолы из этих клеток были изученны. Эти клетки крутятся во вращающемся электрическом поле, которое образуется между четырмя электродами. Ротация, определенная как скорость вращения клетки, отнесенная на квадрат силы поля, в первом приближении не зависит от диаметра клетки. Частота поля, которая индуцирует максимальное вращение клетки, позволяет определить мембранную емкость, или эффективную емкость тех клеток, имеющих твердую клеточную оболочку. В этом случае ионно-обменное поведение клеточной оболочки влияет на скорость вращения. Этот метод чувствительно реагирует на измениние мембранного сопротивления. Ионофоры, включенные в мембрану, целиком угнетают вращение клеток.

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