Cells suspended in a low-conducting medium were exposed to an alternating electric field whose frequency was altered between 1 kHz and 2 MHz. A resonance frequency was observed at which all suspended cells rotated about an axis normal to the field lines (when the electric field strength was larger than a threshold value of about 400 V/cm). This resonance frequency varied from species to species of cells (mesophyll protoplasts of *Avena sativa* = 20–40 kHz, human erythrocytes and ghost cells = 80–100 kHz, yeast cells = 140–180 kHz, Friend cells = 30–40 kHz, at room temperature).

The resonance frequency of cell rotation was observed only under specific experimental conditions which excluded interference by reversible electrical breakdown of cell membranes and by gravitational forces.

Glutaraldehyde fixed and heated cells exhibited no rotation in the frequency and field range investigated.

The phenomenon of rotation is discussed in terms of dipole orientation within the membrane.

**Introduction**

Pohl and Crane (cf. [1, 2]) have reported that yeast cells placed in an alternating electric field spin about an axis normal to the field lines.

Rotation of the cells occurred at a rate of a few revolutions per second, and such spinning was observed at almost any frequency and anywhere in the field when the cells were exposed to an alternating field in a non-electrolyte solution.

However, cell rotation was only observed statistically, i.e. only a few cells were found to be spinning simultaneously at a given frequency and field intensity.

In this communication we report on a resonance frequency at which nearly all cells simultaneously spin in the field. This resonance frequency varies from species to species of cell.

**Materials and Methods**

**Experimental set-up**

Experiments were carried out under the light microscope in a small Perspex chamber with two parallel horizontally-mounted platinum electrodes. (The distance between the electrodes was 100 and 300 μm, respectively, depending on the cell diameter). The temperature was kept at 22 °C. (Fig.1).

The alternating electric field was generated by using a function generator, type TE 7704, Toellner GmbH, Frankfurt, West-Germany. The frequency range investigated was between 1 kHz and 2 MHz.

In order to obtain electrically homogeneous cell populations (see below) the cells were exposed to an electric field pulse of 2 kV/cm intensity and 1 μs duration in a discharge chamber [3].

**Friend cells**

Friend-virus transformed mouse erythroleukemic cells (Friend cells) were obtained by courtesy of Prof. Goebel, University of Würzburg and cultured in a modified Eagle’s basal medium [4].

For the experiments the cells were washed in a solution containing 400 mM/l sucrose. The cells were suspended in a solution containing 20% Percoll and 400 mM/l sucrose and placed between two electrodes of 100 μm distance.

**Baker’s yeast**

Baker’s yeast-cells were washed twice in distilled water prior to the experiment, electrode distance: 100 μm.
Fig. 1. Schematic diagram of the chamber used for the rotation-experiments. Two platinum electrodes mounted on a slide are connected with a function generator (for further details see text).

**Erythrocytes**

Blood cells from apparently healthy donors were washed twice in phosphate buffered saline (to remove the serum and the buffy coat layer) and twice in 300 mM/l glucose. Finally they were suspended in a solution containing 30% Percoll and 300 mM/l glucose. The electrode-gap was 100 μm.

**Erythrocyte-ghosts**

Ghost cells were prepared according to the electrical breakdown method as described elsewhere [5, 6]. Erythrocytes at 4 °C were subjected to an electric field pulse of 8 kV/cm in a Perspex-pulse chamber at a suspension density of 1:20 (packed cells to solution). Resealing of the cells was carried out at 37 °C. The resealed ghost cells were washed twice in 300 mM/l glucose and suspended in a solution containing 30% Percoll and 300 mM/l glucose. The electrode gap was 100 μm.

**Mesophyll-cell protoplasts of Avena sativa**

Protoplasts were obtained from *Avena* leaves by digesting the cell walls enzymatically with cellulysin (Calbiochem., San Diego, USA, for further details, see: [7]). Mesophyll-cell protoplasts were then suspended in a 500 mM/l mannitol solution. The electrode distance was 300 μm.

**Results**

The experimental conditions have to be chosen very carefully in order to observe the resonance spinning of the cells. There are several possible reasons why such a resonance frequency has not been detected earlier by Pohl and coworkers [1, 2] who investigated the phenomenon of cell rotation. Rotation of cells can only be observed above a certain threshold value of the external electric field strength [8]. However, if this field strength exceeds that required to bring about electrical breakdown of the membrane, such a reversible breakdown will occur and result in a dramatic increase in the membrane conductance and permeability [6]. We found that breakdown of the cell membrane led to a cessation of cell spinning. Rotation resumed only after the resealing of the membrane and restoration of the electric properties of the membrane. In addition to abolishing its own ability to rotate, the electrical breakdown of a cell membrane has an influence on the rotation of the other cells in the culture. This is due to two effects. Firstly the escape of cell electrolytes results in an increasing conductivity of the external medium. In consequence local heating occurs, causing turbulence in the medium, thus disturbing the rotation of nearby cells. Secondly, due to the slightly higher pressure inside the cells (i.e. the turgor pressure) water is driven out of the cells when breakdown occurs. This also results in local turbulence which tends to drive the cells apart and again disturbs cell rotation. This effect has been described by several authors [2, 8] for higher electric field strengths; however, they were not aware that the repulsion arises from the breakdown of the cell membrane.

The strength of the external field which results in breakdown is strongly dependent on certain features of the cells which may vary widely.

The rise time of the membrane potential in response to the alternating electric field, for example, may vary considerably throughout a cell culture. Thus at a given field intensity and frequency the critical breakdown voltage may only be reached by some cells. One reason for this is that the required external field strength is (inversely) proportional to the cell radius (spherical cells) or to the length of its long axis (ellipsoidal cells) [6].

The value of the breakdown voltage itself can vary within a cell culture and depends also on the duration of the field (i.e. on the frequency) [9]. The value is decreased by decreasing the frequency.

Considering the field strengths reported in the literature [2, 8] to cause cell rotation, it is immediately evident that a significant proportion of the cells should undergo breakdown at such frequencies and intensities.
The interference of cell-rotation caused by membrane breakdown can be avoided if electrically homogeneous cell populations are used. This requirement is fulfilled to a first approximation by human erythrocytes and ghost cells, but not by cell cultures in general. Growing cell cultures, (Friend cells and yeast cells) are very often (although not always) electrically inhomogeneous [3]. The reason for this variation is not known. Electrical homogeneity can be achieved by subjecting the cell population to an electric field pulse of an intensity and duration which is sufficient to destroy irreversibly the larger cells and the cells which exhibit a low time constant for the membrane-charging process, leaving a residue of cells of similar electrical properties. This can be performed in the pulse chamber. For Friend cells and yeast cells an electric field pulse of 2 kV/cm and 1 μs duration is suitable. After filtration of the cells through a Millipore filter (pore diameter: 10 μm) a cell suspension is obtained which is homogeneous in size and homogeneous in the electrical properties of the membrane.

A second problem which has to be solved before the resonance frequency can be readily observed is the elimination of movement due to gravity. With a few exceptions (e.g. Avena sativa protoplasts), the density of an isotonic non-electrolyte solution does not correspond to the density of the cells. Thus, gravitational forces act in addition to the electric field forces on the cells. This can lead to a cessation of cell rotation. Beside this, it is very difficult to observe the rotation because the cell which is under observation tends to leave the level of focus of the microscope.

The appropriate density was achieved by addition of Percoll to the solution. Since Percoll solutions are normally conductive and have a pH-value of 9, the Percoll solution was pretreated with the ion exchange resin Amberlite MB-1 (Serva GmbH, Heidelberg). Such treated solutions have a pH-value of 7 and show a conductivity of less than $5 \times 10^{-6} \Omega^{-1} \text{cm}^{-1}$. Under these appropriate conditions (see Methods) the cells float at a given level between the electrodes.

If non-uniform breakdown is avoided and the gravitational forces eliminated, it is still difficult to detect the resonance frequency. We have found that, once the threshold electric field strength is exceed-
ed, some time is needed (in the range of seconds to minutes, especially in the case of larger cells like Avena protoplasts) to initiate rotation due to inertia and to the frictional forces exerted on the cells by the external medium. This problem can be overcome by using the dielectrophoresis effect combined with high suspension densities.

Exposure of suspended cells to a non-uniform field as in the electrode arrangement used in this study leads at high frequencies to the formation of "pearl chains" and bridges between the two electrodes (Fig. 2). This effect was termed dielectrophoresis (2) and is caused by the polarisation of the cell in the field. The polarized cell moves towards the region of the greatest field intensity, no matter which electrode is charged positive and which is negative. If the suspension density is high, the "pearl chains" and the bridges will attach to each other.

Removal of the field results in these pearl chains disaggregating. This also occurs when the applied frequency is altered to that required for rotation. However, if the cell density is sufficiently high the cells do not move very far from each other. In this case as some cells begin to rotate, the rotation of adjacent cells can be initiated by mechanical pulse-transfer, by dipole-coupling or by hydrodynamic forces acting in the space between the cells. Provided that the resonance frequency is reached and the threshold field strength is exceeded, then all the suspended cells will rotate. The high suspension density will in fact amplify cell rotation which thus becomes visible under the light microscope. The resonance frequencies were determined for Friend cells, yeast cells, mesophyll protoplast cells of Avena sativa, erythrocytes and ghost cells prepared by electrical haemolysis and shown to be 30–40 kHz, 140–180 kHz, 20–40 kHz and 80–100 kHz, respectively, under the experimental conditions used here.

Below and above this value only a few cells were observed to spin, as described by Pohl [2]. Only in a narrow frequency range, do all cells of the suspension rotate. It should be noted that also single cells close to the electrodes rotated at the resonance frequency. Up to date, we have no clear-cut indication, that an individual free cell can rotate far away from the electrodes in contrast to the findings of Pohl and Crane [1]. The threshold value of the electric field-strength at the resonance frequency for all cell types was between 300 and 500 V/cm.

Above the threshold of the electric field strength the revolving rate increased with the field strength.

Cells which were pretreated by glutaraldehyde (2%, 1h duration) showed no spinning over the whole frequency range between 20 kHz and 2 MHz, even at the maximum voltage amplitude of 20 V (corresponding to a field strength of about 2 kV/cm, depending on the electrode distance used). Only occasionally were some cells found in rotation. However, these very few exceptions might have been cells which were not completely fixed by glutaraldehyde.

It is possible that higher field strengths could induce a resonance spinning of fixed and heated cells. This must be tested in the future when equipment becomes available by which higher electric field strengths and frequencies can be applied. In this context it should be also noted that at these high electric field strengths no electrical breakdown of the fixed cells occurs because cross-linkage of the cell membrane by glutaraldehyde shifts the breakdown voltage to very high values [10]. On the other hand, it is worthwhile to mention that glutaraldehyde-fixed yeast cells can still be arranged in high yield as pearl chains at the electrode surfaces although the maximum voltage which is available by the equipment has to be applied. This result shows that "dead" cells can also exhibit dielectrophoresis at a frequency at which normally positive dielectrophoresis occurs for living cells, providing a higher field strength is used. This result differs from that of Pohl [2] who found that dead cells accumulated in much lower yield at a lower frequency than living cells.

Furthermore, yeast and Friend cells subjected to temperatures of 57 °C for 30 min still show the dielectrophoresis phenomena, however no rotation. A further clear cut indication that the resonance frequency can be attributed to the living cells and that it does not arise artificially comes from observations of the resonance phenomena in a mixed population of two cell types which show different resonance frequencies when investigated alone, e.g., yeast cells (resonance frequency = 140–180 kHz) and Friend cells (resonance frequency = 30–40 kHz). It can be easily shown that the distinct resonance frequencies of these cells when mixed are maintained and that no intermediate frequencies occur. This finding definitely excludes the possibility that thermal ef-
fects or hydrodynamic effects are the primary reason for the rotation.

Further experiments should investigate the dependence of the cell’s resonance frequency on the external conductivity. Such experiments were difficult to perform with the device used in these experiments. At higher conductivities, thermally-induced perturbations occur between the two electrodes due to the increase in current.

**Discussion**

The mechanism of cell rotation is unknown although it will be now possible, because of the all-or-none effect observed, to investigate in more detail the underlying processes. Pohl [11] suggested recently that a dipole is generated in the whole cell by biochemical processes, particularly during the phase of cell division. The finding that human erythrocyte ghost cells still show rotation and exhibit a resonance frequency, however, can only lead to the conclusion that the rotation results from the electrical properties of the membrane or the interface between the membrane surface and the bulk solution.

The further finding that glutaraldehyde-treated and heated cells do not exhibit the resonance phenomena under the experimental conditions used indicates that cell spinning at the resonance frequency seems to be a property of the intact membrane.

A cell would rotate if the frequency of the external field matches the frequency of a natural oscillating dipole located in the membrane and orientated by the electric field. A natural oscillating dipole within the membrane may arise by cooperative periodic translocations of charged proteinacious or lipophilic molecules (carrier molecules) which would lead then to an oscillating, intrinsic field within the membrane. If this hypothesis is true, it would be possible for the first time to get insight into the dynamic changes of the intrinsic electric field within the membrane due to translocation processes. Furthermore, if a natural oscillating dipole exists in the membrane, electromagnetic waves would be emitted, a result which would suggest a new possible means of cellular communication [11, 12].

The hypothesis of a natural, field orientated dipole within the membrane seems to be supported by the value of the turnover frequency of lipophilic ions in solvent free artificial lipid bilayer membranes, which is in the order of 10 kHz [13].

Despite the agreement of this figure with the range of resonance frequency reported here, it cannot be excluded, that rotation occurs due to cooperative orientation of the dipoles of the phospholipids and to some kind of interactions between dipoles generated in two adjacent cells, or due to interactions of the cell with inhomogeneities of the electrode surface. As long as the proof is not established, that a single cell is able to rotate far away from the electrodes, this possible explanation has to be taken into consideration (Holzapfel et al., in preparation).

Since the resonance frequency is different for all cells so far investigated, we can conclude that the membrane properties involved in this effect are different for the various cells. It is therefore of great interest to obtain information concerning the membrane properties that are involved in the rotation of cells. This is particularly true with respect to tumor cells and thus to cancer research.

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