Eur Biophys J 21, 35–45 (1992)

European Biophysics Journal

Formation of cell protrusions by an electric field: a thermodynamic analysis

Michael M. Kozlov [1], Peter I. Kuzmin [2], and Sergey V. Popov [3]

[1] Freie Universität Berlin, Institut für Theoretische Physik, Fachbereich Physik, WE 2, Arnimallee 14, W-1000 Berlin 33, Federal Republic of Germany
[2] A.N. Frumkin Institute of Electrochemistry, USSR Academy of Science, 31 Leninsky Prosp Moscow, 117071, USSR
[3] Columbia University, Department of Biological Sciences, New York, NY 10027, USA

Received April 18, 1991/Accepted in revised form November 27, 1991

Abstract. This work gives a thermodynamic analysis of outgrowth extraction from the cell body by a pulling force. The results are applied for a case when the pulling force is generated by an external high-frequency electric field. Two equilibrium conditions are analyzed: internal equilibrium of an outgrowth and equilibrium between the outgrowth and the cell body. In both cases the stability of feasible equilibrium states was studied. The work shows that the curvature of an outgrowth equilibrated with a pulling electric force depends on the squared amplitude of the electric field E_0^2, on the outgrowth length l and on the transmembrane pressure differential ΔP, and that at a sufficiently large transmembrane pressure differential the cylindrical form of the outgrowth loses its stability. Long outgrowths are more stable than short ones. The minimal value of critical pressure differential was estimated. The work also shows that outgrowth extraction from the cell body requires that the applied force exceeds a critical value below which no outgrowth is formed. The value of the electric field at which outgrowth formation is feasible was estimated.

Key words: Extraction of protrusion – Electric force

Introduction

Most of the cells of an organism are attached to extracellular substrata or to each other with the help of cell protrusions (see Trinkaus 1985 for review). The formation of cell outgrowths has been widely investigated by cell biologists and membrane mechanics researchers (Hochmuth et al. 1973, 1976; Hochmuth and Evans 1982 a, b; Waugh 1982 a, b; Waugh and Hochmuth 1987).

An outgrowth can be extracted from the body of a cell by a mechanical force. One of the early works (Hochmuth et al. 1973) revealed the formation of an outgrowth from the body of a red blood cell placed in a hydrodynamic flow. Subsequently, this phenomenon was studied in detail for red blood cells and lipid vesicles (Hochmuth and Evans 1982 a, b; Waugh 1982 a, b; Waugh and Hochmuth 1987). In the experiments cited the cell was fixed at a point on a support or sucked into an immobile micropipette tip. The external solution was set in motion, the fixed cell being subjected to the frictional force of a hydrodynamic flow. By varying the velocity of the flowing fluid the force tending to carry away the cell could be changed. At a certain magnitude this force extracted an outgrowth from the cell body. The methodology of the experiments made it possible to calculate the outgrowth elongation. The length of the outgrowth obtained in those experiments reached tens of micrometers and the radius was about 20 nm (Waugh and Hochmuth 1987). Such outgrowths are called tethers.

The mechanism of cell protrusion formation was also dealt with in a number of works where the cells were subjected to an external electrical force. As opposed to the other approaches, this method can be applied to spherical cells in suspension (Popov and Margolis 1988) as well as to substrate-attached cells with complex morphology (Margolis and Popov 1991) (e.g. spread fibroplast). It was shown that a membrane-applied force generated in an electric field is sufficient to form morphologically normal cell-specific protrusions. In that case, the authors also succeded in showing that an electric field induces outgrowth formation. Electric field-generated protrusions similar to those formed in normal physiological conditions end with a small sphere whose radius is about 3 times larger than the radius of the outgrowth.

The reports on cell protrusion formation in an electrical field investigated the mechanism of cell outgrowth formation, the factors that determine the morphology and the role of the cytoskeleton in this process. However, the experimental system developed can also be used to study the mechanical properties of the membrane of a "typical" cell with complex cell morphology.

The aim of this work is to describe an outgrowth formed by an electric force. The difference in action of an electric field and a hydrodynamic flow is that, first and foremost, the electric pulling force depends on the outgrowth length. Therefore, it is of interest to determine the

relation between the curvature of an outgrowth and its length, analyze the stability of the cylindrical form of the outgrowth and, finally, find criteria for outgrowth formation. We shall not use specific membrane models and shall use the general description valid for any interface.

Statement of the problem

Consider a cell (vesicle) with an outgrowth formed by a pulling force that can be generated, for instance, by an external electric field. The cell membrane is a bilayer consisting of two lipid molecule monolayers. To describe the mechanical and thermodynamic properties of the membrane, we shall use the formalism offered by Gibbs (1928) and recently developed in a number of works on the physical chemistry of interfaces (Buff 1956; Murphy 1966; Boruvka and Neumann 1977; Kozlov and Markin 1989a, b). Inside the membrane we select a separating surface the normal to which coincides everywhere with that to the membrane surface. All geometrical, mechanical and thermodynamic characteristics shall be, according to the Gibbs' method, referred to the separating surface.

The cell has the form of a sphere of radius R. The outgrowth is a cylinder of length l and section radius ϱ. Below we shall use the concept that membrane outgrowth curvature is equal to $J = 1/\varrho$. The outgrowth end has the form of a spherical surface element of radius r (Fig. 1).

The linear dimensions of various parts of the system considered obey the following relationships. Radius of the cell, R, significantly exceeds all linear dimensions of the outgrowth: $R \gg l, \varrho, r$. In other words, any part of the outgrowth, its spherical end included, is close to the cell surface. The outgrowth itself is strongly extended: its length significantly exceeds the cross section radius, $L \gg \varrho$, and the radius of the terminal sphere, $l \gg r$. Finally, the radius of the spherical end of the outgrowth is much larger than that of its cylindrical part, $r \gg \varrho$.

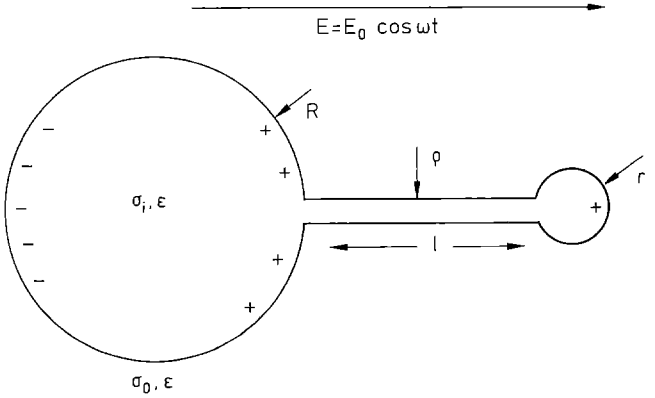


Fig. 1. A cell model with an outgrowth in a uniform electric field. Radius of the cell body, R; radius of the outgrowth membrane curvature, ϱ. At the end of the outgrowth, a spherical bulge of radius r; length of the cylindrical part of the outgrowth, l. Electrical parameters: σ_i, volume conductivity of intracellular medium; σ_0, volume conductivity of washing solution; ε, permittivity of solutions (in all figures taken to equal 80). Specific capacitance of membrane, C_m. The figure schematically shows the distribution of induced charge

Electrical properties of the system

The system is subjected to a high frequency electric field $E = E_0 \cos(\omega t)$. The outgrowth axis is parallel to the field intensity vector E_0. Electrical features of the system are determined by the conductance of the intracellular medium σ_i, and external solution, σ_0 as well as by the properties of the cell membrane and geometrical characteristics. The membrane features low permittivity and possesses the properties of a plane capacitor with a specific capacitance equal to C_m. In addition, the membrane possesses a background conductance σ_m whose value is much smaller than the conductances of solutions, $\sigma_m \ll \sigma_i, \sigma_0$. Consider that the electrical properties of the system satisfy the following relationships. First, the intracellular medium possesses higher conductance than the extracellular solution, $\sigma_i \gg \sigma_0$. Second, the specific membrane capacitance and solution conductivities are such that the characteristic time of membrane charge in the external electric field significantly exceeds that of the external field change, $T = 1/\omega$. In this case the membrane resistance to the alternating current is negligible, the potential drop across the membrane can be considered to be equal to zero and its influence on the electric field distribution can be neglected.

Under the action of the external field the system polarizes. Consider that, because of the high conductance of the external solution, it remains equipotential at any time. This implies that inside the cell the charge is separated and thus the electric potential, generated by the external field and intrinsic polarization of the cell, is the same everywhere inside the system. On the whole, the system remains electrically neutral; however, charge separation leads to an electric force which tends to extract the outgrowth from the cell.

Mechanical properties of the system

The cell resists outgrowth formation. The resistance is caused by mechanical forces that prevent membrane deformation during the extraction. The mechanical stresses occurring in the membrane are called force factors and are attributed to the separating surface. The force factors are interfacial tension γ, bending stress C_1 and torsion stress C_2.

Force factors of the membrane depend on its geometrical characteristics (Kozlov and Markin 1989a). The connections between the changes in force factors and membrane deformation are determined by the elasticity moduli. Consider that the membrane is non-stretchable (tension-compression elasticity modulus is large). In addition, assume that the bending stress is proportional to the membrane curvature, J

$$C_1 = E_{JJ} J. \tag{1}$$

The membrane bending elasticity modulus in expression (1) serves as a proportionality coefficient. The above assumptions as to the membrane elasticity features correspond to the widely known Helfrich model. The sponta-

neous curvature of the membrane introduced in this model is assumed to equal zero.

Consider that, besides the force factors, the model discussed has the transmembrane pressure differential ΔP equal to the difference between the pressure in the intracellular medium, P_i, and that in the external solution, P_0, $\Delta P = P_i - P_0$.

Outgrowth equilibrium

Initially, prior to extraction, the cell membrane is in a state of equilibrium, its thermodynamic properties being determined by intensive parameters: temperature, chemical potential of constituent molecules, interfacial tension, bending and torsion stresses and transmembrane pressure differential. Intensive parameters depend on extensive ones – membrane area and amount of constituent molecules. Consider that during outgrowth extraction the thermodynamic parameters of the cell body membrane do not change. This is possible if the area of the outgrowth membrane is negligible compared with that of the cell body membrane. Under these conditions the membrane of the cell body plays the role of a stable reservoir from which an outgrowth membrane is extracted.

During the extraction of a cylindrical outgrowth by a pulling force, which can be of an electrical nature, the above force factors are generated in its membrane. The values of these factors depend, on the one hand, on the pulling force applied. On the other hand, the outgrowth is extracted from the cell body with constant conditions that depend on neither the size of the outgrowth or the value of the pulling force. Therefore, the question is, first, whether an outgrowth affected by a pulling force can be in a state of internal equilibrium and, second, if equilibrium is possible between the outgrowth and the cell body (lipid reservoir).

Internal equilibrium of an outgrowth is established when the membrane is subjected to mechanical stresses conforming to the value of a force. For a non-stretchable membrane, this process is accociated with small deformations requiring no significant redistribution of the membrane lipid material. Equilibrium between the outgrowth and the reservoir is associated with the lipid flow through the transition zone that connects the outgrowth with the cell. The process is comparatively slow, its rate being determined by membrane viscosity. Consider that the internal equilibrium of the outgrowth occurs significantly more rapidly than that between the outgrowth and the cell body (lipid reservoir). Therefore, we assume that the system is always in a state of partial equilibrium which suggests internal equilibrium of the outgrowth. Equilibrium between the outgrowth and the reservoir conforming to the total equilibrium of the system is reached only under special conditions.

The aim of the work is a thermodynamic analysis of outgrowth extraction from the cell body by a pulling force. We will analyze geometrical and force characteristics of an outgrowth equilibrated with a pulling force and consider the stability of such an equilibrium. We will find the change of free energy of the system, that accompanies outgrowth extraction from the cell body. Based on this, we will analyze the conditions under which outgrowth extraction proves possible. The results of the analysis will be used to investigate the process of outgrowth formation in the case when the pulling force is an electric force generated by an external electric field.

Basic equations

Free energy of the system

Equilibrium conditions are determined from the extreme value of free energy of a system. In the case considered a cell with an outgrowth is under the action of a pulling force that can be of electrical nature, and under the action of a transmembrane pressure differential. Consider the change in free energy of the system, accompanied by that in the thermodynamic parameters of the outgrowth. The total change of free energy contains the following contributions: work against the pulling force dF_N; work against the pressure differential, dF_P; change in free energy of the cell body, dF_c, associated with material exchange between the outgrowth and the reservoir; and, finally, change in free energy of the outgrowth dF_t.

Expressions for the first three contributions to the change in free energy have the following form: work against the pulling force equals

$$dF_N = -N \cdot dl, \tag{2}$$

where N denotes the pulling force acting along the outgrowth axis; dl is the change in length of the cylindrical part of the outgrowth; work of the pressure differential equals

$$dF_P = -\Delta P \cdot dV \tag{3}$$

where ΔP is the pressure differential depending on the cell volume; dV is the change of the outgrowth volume. The change of free energy of the lipid reservoir is equal to

$$dF_c = \mu_c \cdot dn_c \tag{4}$$

where μ_c is chemical potential of lipid molecules in the reservoir and dn_c is the change in the number of lipid molecules in the reservoir.

The change in free energy of the outgrowth membrane appears as (Murphy 1966)

$$dF_t = -S\,dT + \mu_t\,dn_t + \gamma\,dA + C_1\,A\,dJ \tag{5}$$

where S is excessive entropy referred to the separating surface of the outgrowth membrane; T is the absolute temperature; μ_t is the chemical potential of the lipid molecule in the outgrowth; dn_t is the change in the number of lipid molecules in the outgrowth membrane; A is the area of the outgrowth cylindrical part and J is its curvature equal to the inverse radius. The total change in free energy equals

$$dF = -S \cdot dT + \mu_t \cdot dn_t + \gamma \cdot dA + C_1 \cdot A\,dJ - \\ -\Delta P \cdot dV + \mu_c \cdot dn_c - N \cdot dl. \tag{6}$$

Chemical potential of lipid molecules in the outgrowth

Expression (5) determines the change in free energy of the outgrowth membrane, depending on two extensive variables: number of lipid molecules, n_t, and area, A. Thermodynamic integration of this expression (at constant intensive variables T and J) leads to the expression (Eriksson 1980)

$$F_t = \mu_t \cdot n_t + \gamma \cdot A. \tag{7}$$

Differentiation of expression (7) and comparison of the result with (5) yields an equation linking the change in chemical potential of the outgrowth lipid molecules with the changes in interfacial tension of the outgrowth and its curvature

$$d\mu_t = -\frac{a}{2}(d\gamma - C_1 \cdot dJ) \tag{8}$$

where a is the area per lipid molecule; it is assumed that the number of lipid molecules in both monolayers is equal and the temperature of the system is constant. Expression (8) takes into account the fact that the membrane consists of two monolayers of lipid molecules, as a result of which $A/n_t = a/2$. Considering the non-stretchability of the membrane and (1), integration of (8) results in the following formula for the chemical potential of lipid molecules in the outgrowth:

$$\mu_t = \mu_t^0 - \frac{a}{2} \cdot \gamma + \frac{a}{4} \cdot E_{JJ} \cdot J^2 \tag{9}$$

where μ_t^0 is an integration constant.

Condition of equilibrium relative to outgrowth elongation

The change in free energy of the system for a change in the outgrowth length, dl, with other variables constant ($dT = 0$, $dn_t = 0$, $dJ = 0$, $dn_c = 0$) appears, in accordance with (6), as

$$dF = \gamma \cdot dA - \Delta P \cdot dV - N \cdot dl. \tag{10}$$

Taking into account the relation between the area of the cylindrical part of the outgrowth A, its volume V and length l

$$A = \frac{2\pi}{J} \cdot l, \qquad V = \frac{\pi}{J^2} \cdot 1 \tag{11}$$

we obtain from (10) at constant curvature J

$$dF = \left(\frac{2\pi}{J} \cdot \gamma - \frac{\pi}{J^2} \cdot \Delta P - N\right) \cdot dl. \tag{12}$$

The equation for outgrowth equilibrium with the pulling force has the form

$$\gamma = \frac{1}{2\pi} \cdot N \cdot J + \frac{1}{2} \cdot \frac{\Delta P}{J}. \tag{13}$$

Condition of outgrowth equilibrium in radial direction

The change in free energy of the system for a change in the outgrowth curvature dJ with other variables constant ($dT = 0$, $dn_c = 0$, $dn_t = 0$, $dl = 0$) appears, from (6), in the form

$$dF = \gamma \cdot dA + C_1 \cdot A \cdot dJ - \Delta P \cdot dV. \tag{14}$$

Making use of (11) at an outgrowth constant length l, we obtain from (14)

$$dF = -\frac{A}{J^2}(\gamma \cdot J - C_1 \cdot J^2 - \Delta P) \cdot dJ. \tag{15}$$

The equation for outgrowth equilibrium in radial direction has the form

$$\gamma \cdot J - C_1 \cdot J^2 = \Delta P. \tag{16}$$

Expression (16) is a generalized Laplace equation (Murphy 1966; Kozlov and Markin 1989) for a cylindrical surface.

Work of outgrowth extraction from the reservoir

Consider the change in free energy of a system, conforming to outgrowth extraction from the reservoir (cell body). During such a process, the temperature is constant, $dT = 0$; outgrowth curvature does not change, $dJ = 0$. The length of the cylindrical part of the outgrowth changes by dl, the area and the volume changing correspondingly (by dA and dV). In addition, the number of molecules in the outgrowth increases ($dn_t = -dn$) and simultaneously the number of molecules in the reservoir ($dn_c = dn$) decreases. The change in free energy of system (6) takes the form

$$dF = (\mu_t - \mu_c) \cdot dn + (\gamma \cdot dA - \Delta P \cdot dV - N \cdot dl). \tag{17}$$

According to the assumptions made in the previous section, the outgrowth is in equilibrium with the pulling force. Therefore, equilibrium condition (13) is satisfied and the part of the expression (17) in the second parenthesis equals zero. The result is an expression for the free energy change of a system during outgrowth extraction from the reservoir

$$dF = (\mu_t - \mu_c) \cdot dn.$$

The change in the number of molecules during extraction is proportional to that in the outgrowth length,

$$dn = \frac{4\pi}{Ja} dl.$$

Taking into account expression (9) for the chemical potential of the outgrowth lipid molecule, we obtain the final expression for the difference of chemical potentials across the outgrowth and the cell

$$\Delta\mu = \mu_t^0 - \mu_c - \frac{a}{2} \cdot \gamma + \frac{a \cdot E_{JJ}}{4} \cdot J^2. \tag{18}$$

The change in free energy during outgrowth extraction by dl equals

$$dF = \Delta\mu \cdot \frac{4\pi}{Ja} \cdot dl. \tag{19}$$

The value $\mu_t^0 - \mu_c$ has the sense of a standard chemical potential difference, not depending on the parameters of an outgrowth. Below it will be designated as $\Delta\mu^0 = \mu_t^0 - \mu_c$. From expression (18) it follows that equilibrium between the reservoir and the outgrowth occurs if

$$\Delta\mu^0 = \frac{a}{2} \cdot \gamma - \frac{a \cdot E_{JJ}}{4} \cdot J^2, \tag{20}$$

values γ and J being determined from equilibrium Eqs. (13) and (16). If equality (20) is not satisfied, then either outgrowth extraction or suction into the cell body is energetically favourable. Extraction is advantageous for the system if

$$\Delta\mu^0 < \frac{a}{2} \cdot \gamma - \frac{a \cdot E_{JJ}}{4} \cdot J^2. \tag{21}$$

Suction is energetically favourable if

$$\Delta\mu^0 > \frac{a}{2} \cdot \gamma - \frac{a \cdot E_{JJ}}{4} \cdot J^2. \tag{22}$$

Electric force acting on the outgrowth

Consider polarization of our system (spherical cell with an outgrowth) in an external electric field. It should be noted that the electrical properties of the system are such that the presence of the membrane can be neglected and the intracellular solution is considered equipotential at any time. The potential of the intracellular solution can be considered to be equal to zero without any loss of generality.

Under the action of an external field charge separation occurs inside our system (we neglect charge separation in the external solution owing to its small conductance, $\sigma_0 \ll \sigma_e$). Firstly, the body of the cell is polarized. The charges of different polarities diverge to the opposite ends of the cell body. An electric dipole moment is induced on the cell. Secondly, the charge is redistributed between the cell body and the outgrowth. Part of the charge overflows to the spherical bulge at the end of the outgrowth. The cell body receives the same non-compensated electric charge of the opposite sign. Overall, charge redistribution inside the system provides the equipotentiality of the intracellular solution at any given time.

In the bathing solution, charge distribution contains the following contributions: external electric field and the field generated by charge distribution in the cell body. We consider only the dipole component of the cell body potential.

Owing to the small size of the spherical bulge at the outgrowth end, it does not influence electric field distribution, which is determined by the polarization of the cell body in the external electric field. The potential of a small ball at the end of the outgrowth contains the following contributions. First, the potential of the external field at the point of location of the small ball

$$\phi_1 = -E(R+l).$$

Second, the potential generated at distance $l+R$ from the centre of the cell by the dipole moment induced on the spherical cell body

$$\phi_2 = E \cdot R^3/(R+l)^2.$$

Third, the potential of the ball, generated by charge e that appeared at the outgrowth end as a result of charge redistribution

$$\phi_2 = e/4\pi \cdot \varepsilon \cdot \varepsilon_0 \cdot r.$$

In the above formulae, ε_0 is the permittivity of vacuum, ε is the dielectric permittivity. Setting to zero the total potential of the intracellular solution, equal to the sum of these three expressions, makes it possible to find an equation for determining the charge of the ball at the end of the outgrowth

$$-E(R+l) + E \cdot R^3/(R+l)^2 + e/4\pi \cdot \varepsilon \cdot \varepsilon_0 \cdot r = 0. \tag{22}$$

Solution of (22) yields

$$e = 4\pi \cdot \varepsilon \cdot \varepsilon_0 \cdot r(R+l) \cdot E\left[1 - \frac{R^3}{(l+R)^3}\right]. \tag{23}$$

We neglected the disturbance of the cell body electrical neutrality because its consideration gives the correction to charge e in the following order relative to r/l. It should be noted that in (23) the external field E and, along with it, induced charge e depend on time as $\cos(\omega t)$. The force N_{el} acting on the tip of the outgrowth is found by multiplying charge e by electric intensity $E\left[1 + \frac{2R^3}{(l+R)^3}\right]$ at a point of the outgrowth end and averaging the expression obtained in time. Finally, for force N_{el} we obtain

$$N_{el} = 2\pi \cdot \varepsilon \cdot \varepsilon_0 \cdot E_0^2 \cdot r(R+l)\left[1 - \frac{R^3}{(l+R)^3}\right]\left[1 + \frac{2R^3}{(l+R)^3}\right]. \tag{24}$$

This expression is valid to an accuracy of the members of a higher order relative to r/l. In addition, when deducing formula (24) we did not use the assumption that the length of the outgrowth is much smaller than the cell radius, $l \gg R$, because this formula is also valid for an arbitrary relationship between l and R. In the considered case of not very long outgrowths, for which the relationship $\varrho, t \ll l \ll R$ is valid, the expression for N_{el} is simplified to

$$N_{el} = 18\pi \cdot \varepsilon \cdot \varepsilon_0 \cdot E_0^2 \cdot r \cdot l. \tag{25}$$

Expressions (24) and (25) are valid in the case when the outgrowth length l is much larger than the radius ϱ. For very short outgrowths, when $l \simeq \varrho$, formulae (24) and (25) are inapplicable and give incorrect results. As an estimate for the force acting on a very short outgrowth we shall make use of the expression for the force on the part of the electric field on the outgrowth nucleus, which is a semispherical membrane bulge on the cell surface at the point of outgrowth formation. We assume the radius of the

nucleus to be equal to that of the spherical bulge at the end of the outgrowth formed. This force, N_3, is obtained as a result of integration for nucleus surface of the difference of Maxwell stresses about the external and internal membrane surfaces, followed by averaging the results in time

$$N_s = \frac{81}{8}\pi \cdot \varepsilon \cdot \varepsilon_0 \cdot r^2 \cdot E_0^2. \tag{26}$$

As an expression approximately describing the force acting on an arbitrary length outgrowth, we use the sum of (25) and (26); the result is

$$N_{el} = N_s\left(1 + \frac{16}{9} \cdot \frac{l}{r}\right). \tag{27}$$

The expression obtained correctly describes the dependence of an electric force on the length of an outgrowth in the extreme cases of short and long outgrowths. This expression will be used below.

Results

Outgrowth internal equilibrium

Equations (13) and (16) determine the equilibrium of an outgrowth relative to a pulling force N and transmembrane pressure differential ΔP. Consideration of the link between the bending stress and curvature (1) and the joint solution of (13) and (16) leads to an equation for the curvature of an equilibrium outgrowth

$$J^3 - \frac{1}{2\pi} \cdot \frac{N}{E_{JJ}} \cdot J^2 + \frac{1}{2} \cdot \frac{\Delta P}{E_{JJ}} = 0. \tag{28}$$

Equation (28) is similar to the equilibrium expression obtained by Waugh and Hochmuth (1987).

Solution of (28) results in the link between the outgrowth curvature on the one hand and the value of the pulling force on the other hand. The exact solution is bulky. Therefore, we analyze the conditions in which the solution of (28) exists and find an approximate form of the solution. The reasonable physically sensible solutions of (28) are positive.

Depending on the value of the pressure differential ΔP, the following cases are possible. If the transmembrane pressure differential ΔP is absent, $\Delta P = 0$ and (28) has the stable solution

$$J = \frac{1}{2\pi} \cdot \frac{N}{E_{JJ}}. \tag{29}$$

Expression (29) shows that the curvature of the outgrowth membrane increases with the pulling force. A similar relationship was obtained by Waugh and Hochmuth (1987). If the pressure differential is positive and not too large, then two solutions of (28) are feasible, one of which is stable and the other unstable. An approximate form of the stable solution at small differential pressures, $\Delta P \ll E \cdot J^3$, is given by the formula

$$J = \frac{1}{2\pi} \cdot \frac{N}{E_{JJ}} - 2\pi^2 \frac{\Delta P E_{JJ}}{N^2}. \tag{30}$$

The solution (30) reduces to the exact solution of (29) at $\Delta P = 0$. From (30) it is seen that as the transmembrane pressure differential increases, the curvature of the outgrowth, that conforms to its equilibrium relative to the pulling force, decreases.

And, finally, if the pressure differential exceeds the critical value, then the stable positive solution of (28) vanishes. The value of the critical pressure differential equals

$$\Delta P^* = \frac{1}{27\pi^3} \cdot \frac{N^3}{E_{JJ}^2}. \tag{31}$$

If the pressure differential exceeds the value of ΔP^*, the cylindrical form of the outgrowth is unstable.

Substitution of the expression for electric force, (26), into formulae (29)–(31) makes it possible to analyze the curvature of an outgrowth formed by an electric field. In addition, it is of interest to analyze the conditions for outgrowth stability relative to the length of the outgrowth and the intensity of the field.

From expressions (30) and (31) we obtain

$$J = \frac{1}{2\pi} \cdot \frac{N_s}{E_{JJ}}\left(1 + \frac{16}{9} \cdot \frac{l}{r}\right) - 2\pi^2 \cdot \frac{\Delta P E_{JJ}}{N_S^2} \cdot \left(1 + \frac{16}{9} \cdot \frac{l}{r}\right)^{-2} \tag{32}$$

where N_s is determined by (26).

The curvature of the outgrowth (at a given electric field value) increases with length and decreases as pressure differential goes down. These dependences are illustrated in Fig. 2 a–c. At a given value of its length and ΔP the curvature of an outgrowth increases with the amplitude of the external field.

Considering (27), the expression for a critical pressure differential, (31), takes the form

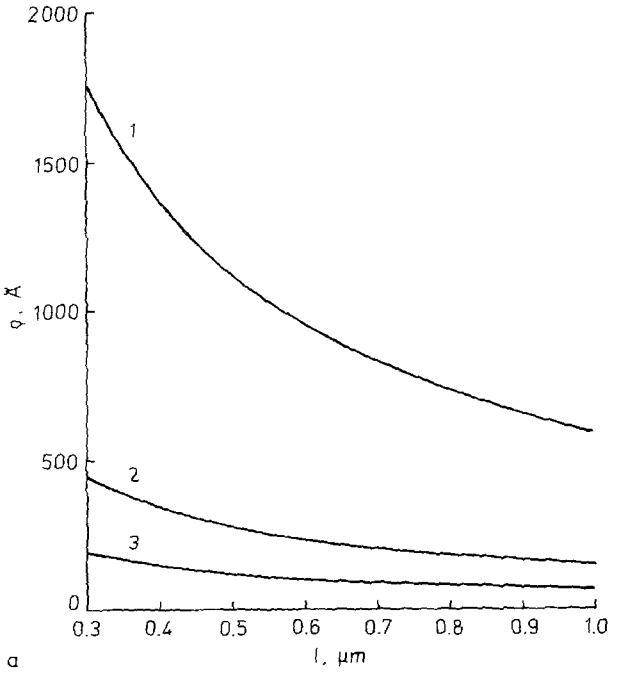
$$\Delta P^* = \frac{1}{27\pi^3} \cdot \frac{N_s^3}{E_{JJ}^2}\left(1 + \frac{16}{9} \cdot \frac{l}{r}\right)^3. \tag{33}$$

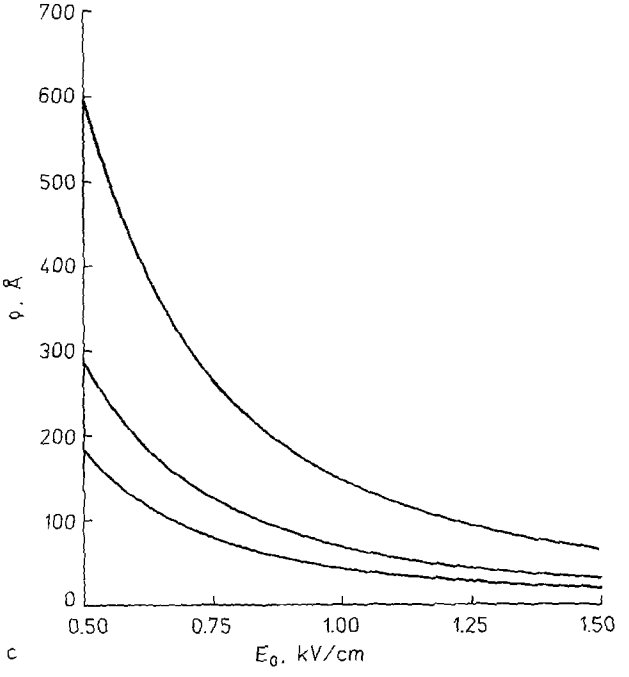
As seen from (33), the critical pressure differential increases with outgrowth length l (Fig. 3). This implies that short outgrowths are less stable than long ones. Elongation of the outgrowth and the associated increase of the pulling force stabilizes the cylindrical form. To obtain a general criterion for the stability of any outgrowths formed in an electric field, we assume length $l = 0$ in expression (33). The result is a lower bound evaluation for the critical pressure differential and has the form

$$\Delta P^{**} = \frac{1}{27\pi^3} \cdot \frac{N_s^3}{E_{JJ}^2}. \tag{34}$$

Figure 4 illustrates the dependence of the critical pressure differential on external electric field intensity. It is seen that the critical pressure differential rises with intensity. In other words, an electric field stabilizes the cylindrical form of the outgrowth.

To proceed further, we shall determine the value of interfacial tension in the outgrowth. From expressions





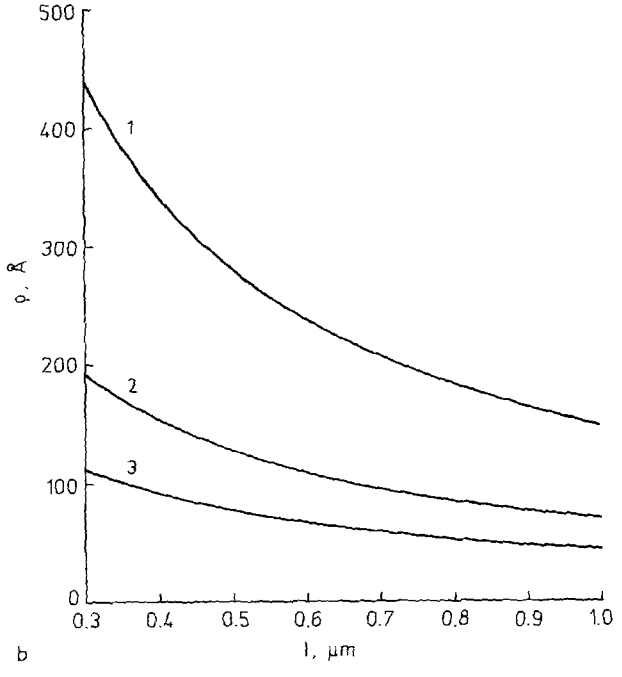


Fig. 2 a–c. Dependence of the curvature radius of the outgrowth cylindrical part, ϱ, on parameters of the model, $E_{JJ}=10^{-19}$ J. **a** dependence of ϱ on l at $r=100$ nm and field intensity values, E_0 (in kV/cm): (*1*), 0.5; (*2*), 1.0; (*3*), 1.5. **b** dependence of ϱ on l at $E_0=1$ kV/cm and values of radius of the spherical end, r (in nm): (*1*), 100; (*2*), 200; (*3*), 300. **c** dependence of ϱ on E_0 at $l=1$ μm; values of parameter r in the curves correspond to those in **b**

(31) and (30) we obtain

$$\gamma = \frac{1}{4\pi^2} \cdot \frac{N^2}{E_{JJ}}. \tag{35}$$

The relation of the interfacial tension to the outgrowth length in the case of an electric pulling force is obtained from (35), (26) and appears as

$$\gamma = \frac{1}{4\pi^2} \cdot \frac{N_s^2}{E_{JJ}} \left(1 + \frac{16}{9} \cdot \frac{l}{r}\right)^2. \tag{36}$$

Tension increases with the length of the outgrowth. Further, we shall assume that the transmembrane pressure differential is equal to zero, $\Delta P = 0$.

Work of outgrowth extraction from the reservoir (cell body)

Considering (35) and (32), expression (18) for the chemical potential difference of lipid molecules in the outgrowth and the cell body takes the following form

$$\Delta\mu = \Delta\mu^0 - \frac{a}{16\pi^2} \cdot \frac{N^2}{E_{JJ}}. \tag{37}$$

Expression (37) shows that equality of chemical potentials of lipid molecules in the outgrowth and the cell body is feasible only at a certain value of the pulling force N^* equal to

$$N^* = \frac{\Delta\mu^0 \cdot 16\pi^2}{a} \cdot E_{JJ}. \tag{38}$$

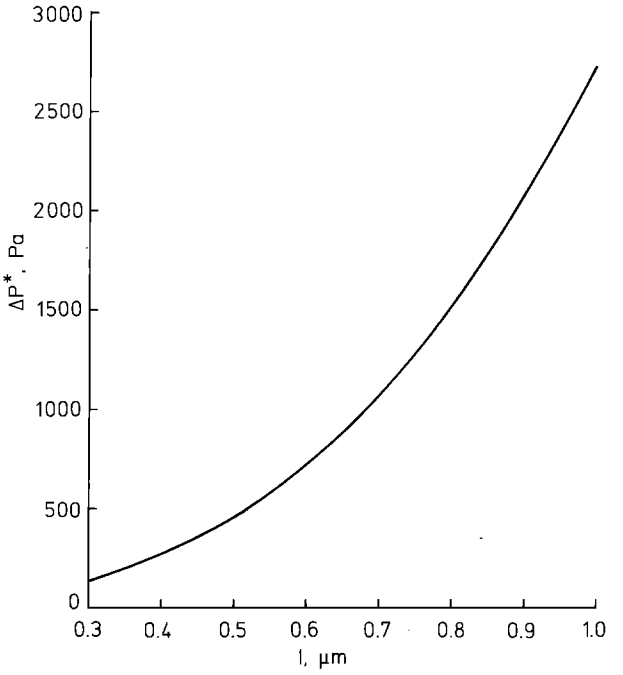


Fig. 3. Dependence of critical pressure ΔP^* on outgrowth length l. Values of parameters: r, 200 nm; $E_{JJ}=10^{-19}$ J, $E_0=1$ kV/cm

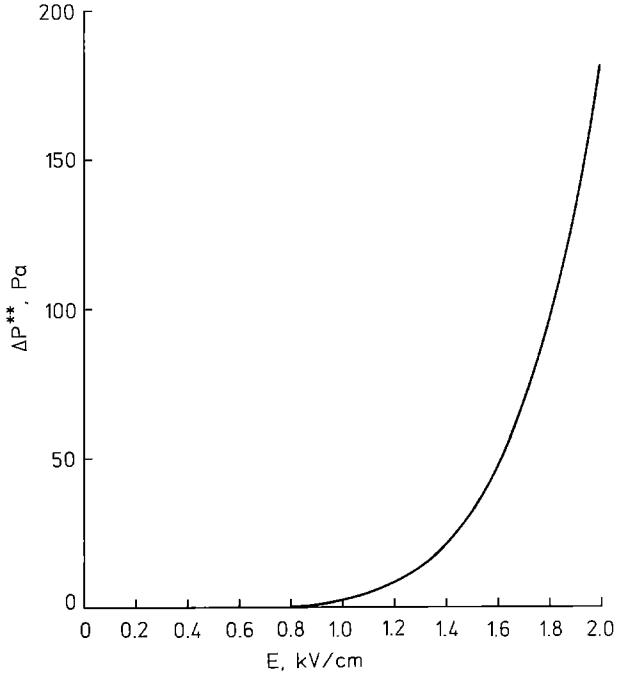


Fig. 4. Dependence of minimal critical pressure ΔP^{**} on amplitude of applied field E_0. Values of parameters are as in Fig. 3

If the magnitude of the pulling force exceeds the critical value, $N > N^*$, then chemical potential in the outgrowth is lower than in the cell body and the outgrowth tends to pull out. In the opposite case, when the pulling force is low as compared with the critical force, $N < N^*$, the chemical potential in the outgrowth is higher than in the reservoir and the outgrowth tends to pull into the body of the cell.

If the pulling force equals the critical force, $N = N^*$, the residue is in a state of total equilibrium. The character of this equilibrium is determined by how the pulling force depends on the outgrowth length.

If the pulling force does not depend on the outgrowth length and its value is critical (38), the outgrowth is in an equilibrium state which is indifferent to elongation. This conclusion is supported by the experiments of Waugh (1982 b) where the force extracting an outgrowth from the vesicle was generated by a hydrodynamic flow and did not depend on the outgrowth length. The work cited showed that the state in which the outgrowth does not change its length with time exists only at a definite rate of the flowing fluid, that determines the corresponding value of the extracting force. At other values of fluid velocity the outgrowth either was extracted or sucked into the vesicle. The outgrowth not changing with time could be of any length. This implies that such an outgrowth was in a state of indifferent equilibrium.

If the outgrowth is extracted by an electric field, the situation occurring differs from the case above. The pulling force generated by the electric field increases with outgrowth length (27). The difference of chemical potentials (37) can become zero only at a definite critical outgrowth length l^* conforming to the critical value of the force, $N_{el}=N^*$ and provided by equation

$$N^* = N_s\left(1 + \frac{16}{9}\cdot\frac{l^*}{r}\right).$$

The outgrowth critical length l^* conforms to the equilibrium of the system, unstable relative to extraction from the reservoir. Indeed, if the outgrowth length exceeds l^*, the outgrowth tends to pull out without bound and move away from the equilibrium position. The change of free energy in this case is negative.

For short outgrowths with precritical lengths, $l < l^*$, the pulling electric force is smaller than the critical value, $N_{el} < N^*$ and the outgrowth tends to pull into the reservoir. Extraction of a precritical outgrowth is associated with an increase in free energy of the system.

Considering (19), (37) and (38), the change in free energy of the system, conforming to outgrowth extraction (18) appears as

$$\mathrm{d}F = \frac{1}{2}\cdot\frac{(N^{*2}-N^2)}{N}\cdot \mathrm{d}l. \tag{39}$$

Integration of (39) results in the expression

$$F = F_0 + \frac{N_s r}{2}\left[\frac{9}{16}\left(\frac{N^*}{N_s}\right)^2 \ln\left(1+\frac{16}{9}\cdot\frac{l}{r}\right) - \frac{8}{9}\cdot\left(\frac{l}{r}\right)^2 - \frac{l}{r}\right] \tag{40}$$

where F_0 is the integration constant.

The dependence of the free energy of the system on outgrowth length, $F(l)$, is illustrated in Fig. 5. As seen, outgrowth extraction is associated with the overcoming of an energy barrier. The barrier is due to extraction of a precritical outgrowth. Its peak corresponds to the outgrowth critical length l^* at which the pulling force has the

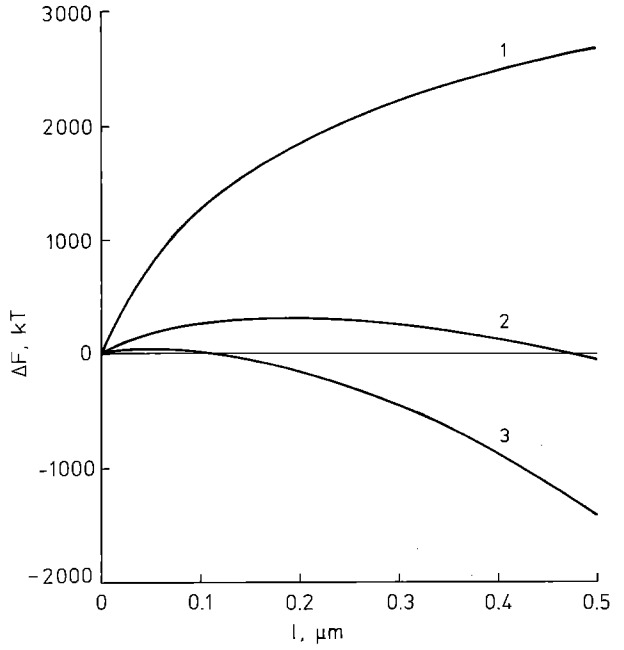


Fig. 5. Free energy of outgrowth extraction (in $kT = 4 \cdot 10^{-21}$ J) depending on length of the cylindrical part, l. $N^* = 10^{-11}$ N, $E_{JJ} = 10^{-19}$ J, $r = 10$ nm. Amplitude of electric field (in kV/cm): (*1*), 0.5; (*2*), 1.0; (*3*), 1.5

critical value N^*. The state at the barrier peak conforms to the equality of chemical potentials of the outgrowth and cell body molecules and corresponds to the unstable equilibrium discussed above. The value of the energy barrier is shown to equal to

$$\Delta F = \frac{9}{64} \cdot N_s r \left[\left(\frac{N^*}{N_s} \right)^2 \ln \left(\frac{N^*}{N_s} \right)^2 + 1 - \left(\frac{N^*}{N_s} \right)^2 \right] \quad (41)$$

where N^* is given by expression (38) and N_s is determined in (26). Figure 6 illustrates the dependence of the energy barrier height on the amplitude of the external electric field. The energy barrier is seen to diminish with the increase of the electric field amplitude, F_0, and becomes zero at $N_s = N^*$, which conforms to

$$E_0 = \left(\frac{4}{9} \cdot \frac{N^*}{\pi \cdot \varepsilon \cdot \varepsilon_0 \cdot r^2} \right)^{1/2}. \quad (42)$$

An increase in the value of the barrier at $N_s > N^*$ yielded by formula (41) conforms to non-physical situations (negative values of critrical length l^*).

Discussion

The work described here gives a thermodynamic analysis of outgrowth extraction from the body of a cell by a pulling force. The results are applied for a case when the pulling force is generated by an external high-frequency electric field.

Predictions for experiment

Results obtained from the above thermodynamic analysis of outgrowth formation make possible some experimentally checkable predictions.

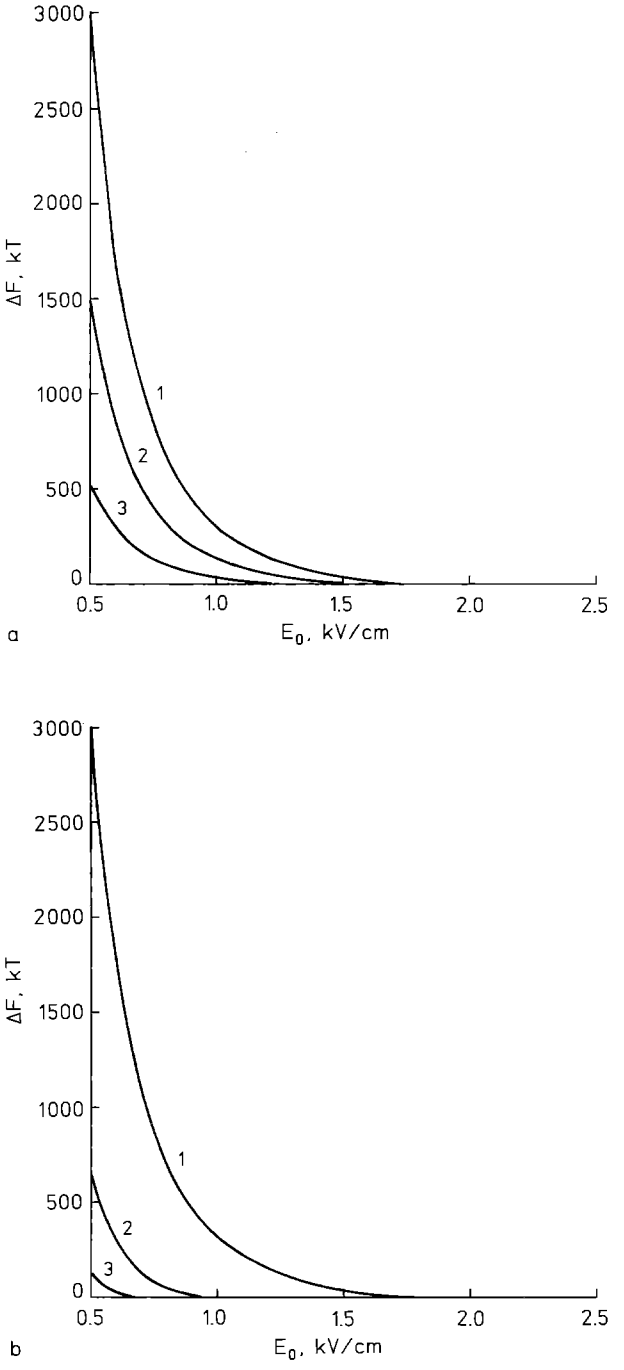


Fig. 6 a, b. Dependence of height of outgrowth formation energy barrier (in $kT = 4 \cdot 10^{-21}$ J) on amplitude of electric field intensity E_0. **a** $r = 100$ nm; values of critical force N^* (in N): (*1*), $1 \cdot 10^{-11}$; (*2*), $0.75 \cdot 10^{-11}$; (*3*), $0.5 \cdot 10^{-11}$. **b** $N = 1 \cdot 10^{-11}$ N; values of spherical bulge radius, r (in nm): (*1*), 100; (*2*), 200; (*3*), 300

The work has analyzed two equilibrium conditions: internal equilibrium of an outgrowth and that between the outgrowth and the cell body. In both cases, we investigated the stability of the feasible equilibrium states. The two equilibrium conditions obey the hierarchy due to the fact that the equilibria between the outgrowth and the pulling force are established significantly more rapidly than between the outgrowth and the reservoir. Therefore, within the framework of the model considered the equilibrium between the outgrowth and the pulling force had

priority. Indeed, consideration of a possible equilibrium between the outgrowth and the reservoir has sense only when the outgrowth does exist, i.e., is in a state of stable internal equilibrium relative to the pulling force. Let us consider the results obtained in accordance with this hierarchy.

From expression (32) and considering (26) it follows that the curvature (equal to the inverse radius of the cylindrical cross section) of an outgrowth equilibrated with a pulling electric force depends on the squared amplitude of the electric field E_0^2, outgrowth length l and transmembrane pressure differential ΔP. Formula (32) was obtained for a case of small pressure differential, $\Delta P \ll E_{JJ} J^3$. It implies that the outgrowth curvature increases with field intensity E_0 (at a fixed length) and with length l (at a fixed field intensity). Besides, as seen from (32), the outgrowth curvature diminishes with an increase in transmembrane pressure differential ΔP.

It is important, however, to have in mind that the predictions pertain to an outgrowth equilibrated with the pulling electric force. In an experiment (Popov and Margolis 1988), upon being pulled out the outgrowths attached to a support and the electron-microscopical measurements were done on the attached outgrowths. Upon attachment, the force acting on an outgrowth from the side of the support can differ from the electric force pulling out the outgrowth. Therefore, the curvature of its membrane can change. Probably, however, the cytoskeleton, entering the outgrowth during its pulling out, fixes the membrane curvature. If so, then one hopes that after attachment to the support the membrane curvature remains the same as during the attachment.

The above work shows that at sufficiently large transmembrane pressure differential the cylindrical form of an outgrowth loses its stability. If the pulling force is of an electrical nature, the expression for critical pressure differential causing the stability loss is given by formula (33), considering (26). As seen from (3), long outgrowths are more stable than short ones. Estimation of the minimal value of critical pressure differential is given by formula (34), considering (26). Dependence of the critical pressure differential on the outgrowth length and external field intensity is illustrated in Figs. 3, 4. The effect of the stability loss of a cylindrical outgrowth upon an increase in transmembrane pressure differential as well as the relation of ΔP^* to the electric field amplitude is also of interest for experimental studies.

Let us turn to the interaction between the outgrowth and the reservoir (cell body). A result of this interaction is that during outgrowth extraction the system overcomes an energy barrier (Fig. 5). The value of the barrier is given by expression (41) and depends on the amplitude of the electric field E_0 (Fig. 6). Besides, the formula for the energy barrier, (41), includes N^* – the critical value of the pulling force determined by the difference of standard chemical potentials of lipid molecules across the outgrowth and the cell body. This value can be estimated based on experimental data obtained by Waugh (1982b). If the pulling force does not depend on the outgrowth length, the system is in indifferent equilibrium at $N = N^*$. In the experiments by Waugh (1982b) the pulling hydrodynamic force did not depend on the outgrowth length and the state of indifferent equilibrium was observed at N approximately equal to 10^{-11} N. Approximately the same value of pulling force, leading to indifferent equilibrium of a red cell outgrowth extracted in a hydrodynamic flow, was obtained in experiments by Hochmuth et al. (1976). The plot of the dependence of the energy barrier on the electric field amplitude (Fig. 6) used the values of N^* close to 10^{-11} N. The barrier becomes equal to zero at $N_s = N^*$. It should be noted that the value of the energy barrier at a given field intensity, E_0, depends strongly on the value of the critical force (Fig. 6a), which can only approximately be estimated based on the existing experimental data. Besides, the value of the barrier strongly depends on the radius of the outgrowth spherical terminal, r (Fig. 6b). If the value of the critical force N^* equals 10^{-11} N and the radius of the spherical end is 200 nm, then at an electric field intensity of $E_0 = 100$ kV/m the barrier already vanishes.

Approximations used in the model

The outgrowth is extracted from the cell body which serves as the reservoir of lipid molecules. The reservoir characteristics do not depend on the outgrowth length. This feature of the model was based on an assumption that the area of the outgrowth is negligible as compared with the area of the membrane. Indeed, the area of an outgrowth with a radius of 100 nm and a length of several micrometers is less than 1% of the area of the erythrocyte, which is the smallest cell with the fold-free membrane.

The chemical potential of lipid molecules in the reservoir does not change. Within the framework of this assumption, the shape of the cell is not essential for the further thermodynamic analysis of outgrowth formation. It may be a red cell with a smooth membrane, an epithelial or any other cell whose membrane is folded or else a large spherical lipid vesicle. The assumption of the spherical form of the cell body, made in this work, is necessary only to calculate the electric force extracting the outgrowth.

The description of the mechanical properties of the outgrowth assumed that force factors (interfacial tension, bending stress) in the membrane are isotropic and the internal medium, filling the outgrowth, produces no elastic resistance to elongation. It implies that, first, the outgrowth membrane is devoid of membrane skeleton and, second, the cytoskeleton in the outgrowth is either absent or it passively adjusts to the change in the form of the outgrowth during its extraction. The hypothesis of the passive role of the cytoskeleton during outgrowth elongation was indirectly supported by the results of experiments of Popov and Margolis (1988).

Calculation of electric force

To obtain the expression for electric force acting on an outgrowth, we used two approximations. We considered, first, the intracellular solution to be equipotential and,

second, the membrane capacity C_m and frequency ω of the external field to be sufficiently large to neglect the presence of the cell membrane in calculations of the electric field. Admissibility of such assumptions is determined by the relationships between the period of external field oscillations and characteristic times of various relaxation processes. The latter include the charge relaxation inside the solution in the cell body, inside the outgrowth and inside the spherical bulge at the end of the outgrowth as well as the charging of the cell membrane, the cylindrical part of the outgrowth and its spherical part.

The characteristic time of charge relaxation inside the cell, τ_0, can be estimated as $\varepsilon \cdot \varepsilon_0/\sigma_i$, which for the typical values $\varepsilon = 80$, $\sigma_i = 1\,\mathrm{S/m}$ is $\tau_0 = 10^{-9}\,\mathrm{s}$. Hence, we conclude that the intracellular solution is, indeed, equipotential at external field frequencies less than 10^9 Hz.

To neglect the presence of membrane in the calculation of electric effects, the characteristic time of the external field change should be much less than that of membrane charging. Let us estimate the characteristic times of cell membrane charging for various regions. The cell body membrane is charged via two solutions: intracellular with conductance σ_i and extracellular with conductance σ_0, the limiting factor in the process being the resistance of the extracellular solution which is much greater than that of the intracellular one. The membrane of the outgrowth spherical part is charged similarly. Valid for the characteristic times of charging the membranes of the cell body, τ_1, and outgrowth end, τ_2, are the estimations

$$\tau_1 = \frac{C_m R}{\sigma_0} \gg \tau_2 = \frac{C_m r}{\sigma_0}.$$

For the typical values $C_m = 10^{-2}\,\mathrm{F/m^2}$, $r = 10^{-7}\,\mathrm{m}$ and $\sigma_e = 10^{-4}\,\mathrm{S/m}$ we obtain $\tau_2 = 10^{-5}\,\mathrm{s}$. Hence, the limitation on the external field frequency $\omega \gg 1/\tau_2 = 10^5\,\mathrm{Hz}$.

Let us now estimate the characteristic times of charge relaxation on the membrane of the outgrowth cylindrical part. Here, the membrane is also charged via two solutions, internal and external. Despite the fact that the specific conductivity of the internal solution exceeds that of the external solution be several orders, the effective resistance of the internal part of the outgrowth can be higher than that of the external solution owing to the small thickness of the outgrowth. For the characteristic time of charging the membrane in the cylindrical part of the outgrowth, the following estimations are valid: for the internal part

$$\tau_3 = C_m l^2/\sigma_i \cdot \varrho$$

and for the external part

$$\tau_4 = C_m \varrho \ln(l/\varrho)/\sigma_0.$$

When the outgrowth membrane is charged via the internal solution, $\tau_3 \gg \tau_4$, the equality of potentials of the cell body and the outgrowth spherical end is disturbed. Thus, a valid approximation of the equipotentiality of the intracellular contents is subject to the inequality

$$\frac{\tau_4}{\tau_3} \cdot \frac{\sigma_i}{\sigma_0} \cdot \left(\frac{\varrho}{l}\right)^2 \ln\left(\frac{l}{\varrho}\right) \gg 1 \qquad (43)$$

i.e., the outgrowth should be not very long. For the characteristic values $\sigma_i = 1\,\mathrm{S/m}$ and $\sigma_0 = 10^{-4}\,\mathrm{S/m}$ we obtain the limitation on the length of the outgrowth

$$l \gg 10^2 \varrho = 10\,\mu\mathrm{m} \qquad (44)$$

and on the frequency of the external field

$$1/\tau_4 = 1/\tau_2 = 10^{-5}\,\mathrm{Hz} \ll \omega. \qquad (45)$$

We can finally that the approximations made in calculations of the electric force are valid within the range of frequencies $100\,\mathrm{kHz} \ll \omega \ll 1\,\mathrm{GHz}$ for not very long outgrowths, $l \ll 100$, $\varrho = 10\,\mu\mathrm{m}$.

Acknowledgement. We are grateful to Yu. A. Chizmadzhev and V. F. Pastushenko for fruitful discussions.

References

Boruvka L, Neumann AW (1977) Generalization of the classical theory of capillarity. J Chem Phys 66:5464–5476

Buff FP (1956) Curved fluid interfaces. I. The generalized Gibbs-Kelvin equation. J Chem Phys 25:146–153

Eriksson JC (1980) Thermodynamics of bilayer lipid membranes. In: Rusanov AI, Goodrich FC (eds) The modern theory of capillarity. Khimiya Pblishers, Leningrad

Gibbs JW (1928) The collected works of Josia Willard Gibbs. Longmans, Green and Co, London

Hochmuth RM, Mohandas N, Blackshear Jr PL (1973) Measurement of the elastic modulus for red cell membrane using a fluid mechanical technique. Biophys J 13:747–762

Hochmuth RM, Evans EA, Colvard DF (1976) Viscosity of human red cell membrane in plastic flow. Microvasc Res 11:155–159

Hochmuth RM, Evans EA (1982a) Extensional flow of erythrocyte membrane from cell body to elastic tether. I. Analysis. Biophys J 39:71–81

Hochmuth RM, Wiles, HC, Evans EA, McCown JT (1982b) Extensional flow of erythrocyte membrane from cell body to elastic tether. II. Experiment. Biophys J 39:83–89

Inoue S, Tilney LG (1982) The acrosomal reaction of *Thyone* sperm. I. Changes of the sperm head visualized by high resolution video microscopy. J Cell Biol 93:387–398

Kozlov MM, Markin VS (1989a) Definition of force factors for an interface with non-uniform curvature. J Chem Soc Faraday Trans 2 85:261–276

Kozlov MM, Leikin SL, Markin VS (1989b) Elastic properties of interfaces. Elasticity moduli and spontaneous geometric characteristics. J Chem Soc Faraday Trans 2 85:277–293

Murphy CL (1966) Thermodynamics of low tension and highly curved interfaces. Ph.D. Thesis. University of Minnesota, Engineering, Chemical

Oster GF, Perelson AS (1987) The physics of cell motility. J Cell Sci (Suppl) 8:35–54

Popov SV, Margolis LB (1988) Formation of cell outgrowths by external force: a model study. J Cell Sci 90:379–389

Trinkaus JP (1985) Protrusive activity of the cell surface and the initiation of cell movement during morphogenesis. Exp Biol Med 10:130–173

Waugh RE (1982a) Surface viscosity measurements from large bilayer vesicle tether formation. I. Analysis. Biophys J 38:19–27

Waugh RE (1982b) Surface viscosity measurements from large bilayer vesicle tether formation. I. Experiments. Biophys J 38:29–37

Waugh RE, Hochmuth RM (1987) Mechanical equilibrium of thick, hollow, liquid membrane cylinders. Biophys J 52:391–400