Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

Migrating Cells: Living Liquid Crystals

Hans Gruler [a b], Manfred Schienbein [a], Kurt Franke [a] & Anne de Boisfleury-chevance [b]
[a] Biophysics Department, University of Ulm, Ulm, Germany
[b] Centre d'Ecologie Cellulaire and INSERM U313, Hôpital de la Salpétrière, Paris, France
Version of record first published: 23 Sep 2006.

To cite this article: Hans Gruler , Manfred Schienbein , Kurt Franke & Anne de Boisfleury-chevance (1995): Migrating Cells: Living Liquid Crystals, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 260:1, 565-574

To link to this article: http://dx.doi.org/10.1080/10587259508038729

Mol. Cryst. Liq. Cryst. 1995, Vol. 260, pp. 565–574
Reprints available directly from the publisher
Photocopying permitted by license only

Migrating Cells: Living Liquid Crystals

Hans Gruler*‡, Manfred Schienbein*, Kurt Franke*, Anne de Boisfleury-Chevance‡
* Biophysics Department, University of Ulm, Ulm, Germany
‡ Centre d'Ecologie Cellulaire and INSERM U313, Hôpital de la Salpétrière, Paris, France

Abstract: Two conditions are necessary to built up a condensated state out of elementary units: (i) interaction between the elementary units and (ii) motion of the elementary units. A new type of liquid crystal is found by using migrating cells (human granulocytes) as elementary units. These cells show no interaction or a weak repulsion at high calcium concentration (2.5 mM). But at low calcium concentration ($< nM$) the cells attract each other and form a polar nematic liquid crystal. First, the machine for the directed movement is investigated phenomenologically. The direction of migration is guided by a cellular automatic controller. It can be characterized by two machine coefficients: (i) the response of the automatic controller is proportional to the strength of the extracellular guiding field times a cellular machine coefficient, and (ii) the random movement is induced by stochastic processes in the cellular machinery. These cellular stochastic processes are equivalent to the thermal motion of inert particles. The analogy to the Boltzmann statistics is evident. In the next step, the cell-cell communication is investigated. During one machine cycle, the cell can release metabolic molecules which attract other cells via chemotaxis. Galvanotaxis is another mechanism for the cell-cell interaction since every cell is surrounded by an electric field. The distance dependent cell-cell interaction is determined from the pair correlation function.

Introduction

The process by which particles like molecules, biological cells, etc. exchange information constitute one of the most intriguing areas of biology and of physics [1, 2]. The physical processes of reducing a gas or vapor with freely moving molecules to a liquid, liquid-crystalline or solid form are known to a large extend. Likewise the interaction processes of reducing the state of freely migrating cells to a condensated state are less known. The following processes discussed in literature [3, 4] are as follows: (i) Adhesion or cohesion: The molecular forces in the area of contact between migrating cells act to hold them together. This effect is less important in the discussed situation since the cells attracted each other even without direct cell-cell contact. (ii) Directed movement in an extracellular guiding field: Every cell can transmit signals and other cells can guid their movement by the received signals (chemotaxis for chemical signals and galvanotaxis for electrical signals). Hence, migrating cells have the possibility to form a condensated state (Fig. 1). In the next section, the

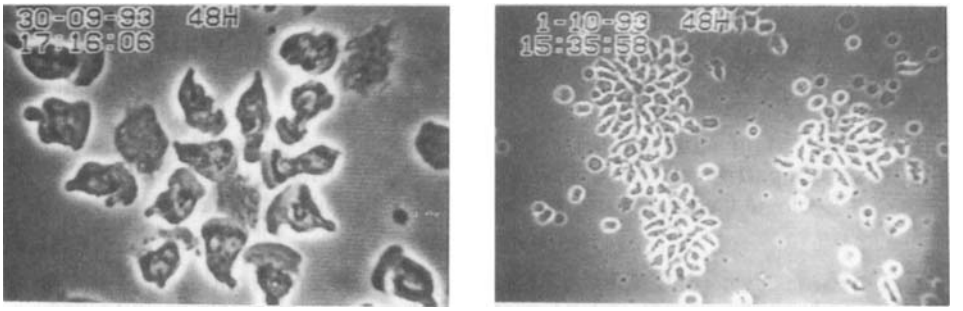

Figure 1: *The cluster formation of migrating human granulocytes exposed to low calcium concentration is shown on two different magnifications. With the picture taken at high magnification, we like to show the cellular attraction towards the cellular cluster. The low magnification picture shows that nearly all cells are concentrated in clusters.*

machine equation for the directed movement is discussed.

The Cellular Automatic Controller

The ability of cells to performe directed movement is due to the existence of a cellular automatic pilot. The machine equation for the angle of migration, φ, is a stochastic differential equation as previously shown [5, 6, 7]

$$\frac{d\varphi}{dt} = -k_P \cdot c_1(field) \cdot sin\varphi + \Gamma_\varphi(t) \tag{1}$$

φ is the angle between the direction of migration and the extracellular guiding field of the strength, $c_1(field)$. The first term on the right side of this equation has the meaning of a deterministic 'torque' which tries to render the movement parallel to guiding field. The machine coefficient, k_P, characterizes the 'deterministic' part of the signal/transduction system of the cellular machinery. The second term, Γ_φ, is a stochastic 'torque' which is responsible for the random walk activity of the locomotory machinery. A comparison between inert particles exposed to an external force and cellular machines exposed to a guiding field is given in [8].

In case of an isotropic cellular environment there exists no desired direction and no guiding field ($c_1(field) = 0$) and, thus, the angle of migration, φ, is only determined by the stochastic torque, $\Gamma_\varphi(t)$. By knowing the stochastic properties of $\Gamma_\varphi(t)$, Eq.1 can be solved. The stochastic term, $\Gamma_\varphi(t)$, can be approximated by a white noise source ($\langle\Gamma_\varphi(t)\rangle = 0$ and $\langle\Gamma_\varphi(t) \cdot \Gamma_\varphi(t')\rangle = q_\varphi \cdot \delta(t - t')$). q_φ is a further machine

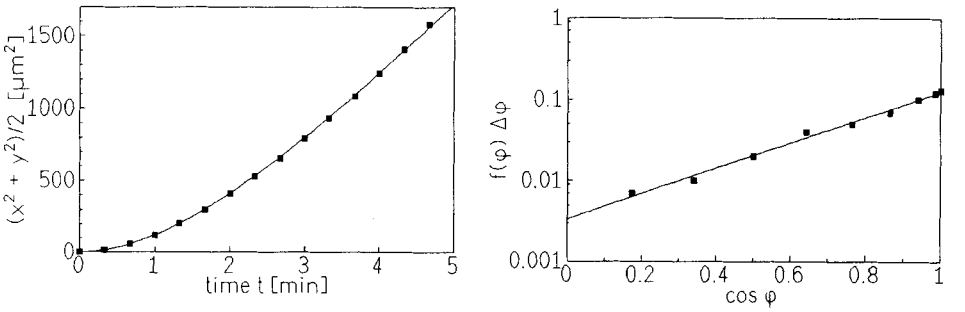

Figure 2: *Left side (a): The mean-squared displacement, $(\langle x^2\rangle+\langle y^2\rangle)/2$, as a function of time, t (6 cells, 116 starting positions). The fit yields $D = 260\ \mu m^2/min$ and $\tau_\varphi = 1.8$ min. Right side (b): The logarithme of the normalized angle distribution function (guided by an electric field) as a function of cos φ.*

coefficient which is related with the persistence time, τ_φ, of the locomotory machinery ($\tau_\varphi = 2/q_\varphi$).

The mean-squared displacement as a function of time can be calculated from Eq. 1 [9] since the cellular speed, $v_c(t)$, and the angle of migration, $\varphi(t)$, are statistically independent variables [10].

$$\langle \Delta x^2 \rangle = \langle \Delta y^2 \rangle = 2D\{t - \tau_\varphi[1 - exp(-\frac{t}{\tau_\varphi})]\} \tag{2}$$

The persistence time, τ_φ, and the diffusion coefficient, D ($= \langle v_c^2 \rangle / q_\varphi$) are obtained by fitting Eq. 2 to the experimental data (Fig. 2a). This equation holds only if there is no cell-cell interaction. Hence, in case of low calcium, the experiments must be performed at low cell density.

In case of a non-vanishing guiding field, $c_1(field) \neq 0$, the random movement of granulocytes became directed. The machine coefficient, k_P, of Eq. 1 can be determined by quantifying the directed movement. The Fokker-Planck equation for the angle distribution function, $f(\varphi, t)$, is in case of a white noise source

$$\frac{\partial f(\varphi,t)}{\partial t} = \frac{\partial}{\partial \varphi}\{k_P \cdot c_1 \cdot sin\varphi + \frac{q_\varphi}{2}\frac{\partial}{\partial \varphi}\} f(\varphi, t) \tag{3}$$

One obtains for steady state ($\partial f/\partial t = 0$) the normalized angle distribution function, $f(\varphi)$, where I_0 is a hyperbolic Bessel function.

$$f(\varphi) = \frac{exp\{\frac{2k_P \cdot c_1}{q_\varphi} \cdot cos\varphi\}}{2\pi \cdot I_0(\frac{2k_P \cdot c_1}{q_\varphi})} \tag{4}$$

The predicted distribution is actually verified by the experiment (Fig. 2b). The dimensionless quantity $2k_P \cdot c_1/q_\varphi$ measures the guiding field in natural units. In case

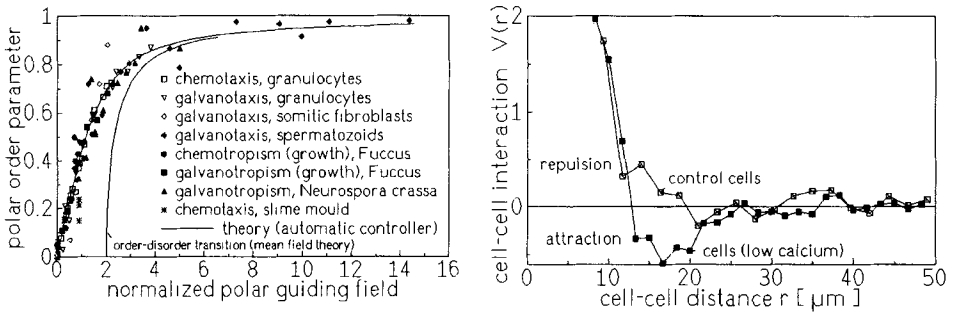

Figure 3: *Left side (a): The dose-response curve of cells guided by exctracellular fields. Different cell types and different guiding fields were used. The line is the prediction in case of a proportional controller. The dashed line describes the predicted order-disorder transition in case of interacting cells. The extraellular polar guiding field, c_1, in Eq. 5 is replaced by the polar mean field, W (Eq. 7). Right side (b): The normalized cell-cell interaction of granulocytes is shown.*

of an electric guiding field, c_1 is the electric field strength, E, but the dimensionless unit is $K_G \cdot E$ with $K_G = 2k_P/q_\varphi$[10]. In case of chemotaxis, the polar guiding field, $c_1(field)$, is the concentration gradient of chemotactic active molecules ($c_1(field) = (1/C)(dC/dx)$). The machine coefficient, k_P, is a function of the cellular detection system: $k_P = k_P^0 \cdot (c')/(1 + c')^2$ where the concentration, C, is measured in cell specific units, $c' = C/K_R$ (K_R is the binding constant of the chemoattractant to the membrane-bound receptor and k_P^0 is a constant).

The directed cellular response can be quantified by the polar order parameter, $\langle cos\varphi \rangle$. The prediction dose-response curve is

$$\langle cos\varphi \rangle = \frac{I_1(\frac{2k_P \cdot c_1}{q_\varphi})}{I_0(\frac{2k_P \cdot c_1}{q_\varphi})} \tag{5}$$

The polar order parameter equals zero for random movement and one if the cells move parallel to the guiding field. Typical results are shown in Fig. 3a. The main result is that different cell types exposed to different guiding fields can be described by an automatic controller connected to a noise source.

Let us summerize this part: The response of a migrating cell exposed to an extracellular guiding field can be described by a stochastic differential equation. The physical basis of this equation is a cellular automatic pilot (proportional controller) which is connected to an intracellular noise generator. The statistical properties of state variables like the angle of migration, are described by an exponential function in analogy to Boltzmann statistics: The numerator is the integrated deterministic signal in the automatic controller and the denominator is the strength of the cellular stochastic process.

The Cell-Cell Interaction

Migrating granulocytes are ideal test cells for the investigation of cell-cell interaction since the behaviour of granulocytes exposed to extracellular guiding fields is known to a large extend [5, 6, 11].

The cell-cell communication of human granulocytes can be altered reversibly by means of the bivalent ions like calcium and magnesium [12]. At high calcium concentrations ($2.5\,mM$), the migration behaviour of a single granulocyte was not affected by other granulocytes except at direct cell-cell contact (steric exclusion). But at low calcium concentrations ($< nM$), the cell behaviour altered: granulocytes attracted each other and formed clusters (Fig. 1).

The cell-cell interaction can be quantified by measuring the pair correlation function, $g(r)$. It is always a positive quantity and can, thus, be expressed by a new function - the generating function, $V(r)$ [1].

$$g(r) = e^{V(r)} \tag{6}$$

This new defined function, $V(r)$, is zero for non interacting cells. The cell-cell interaction is attractive for $V(r) > 0$ and repulsiv for $V(r) < 0$. The generating function approaches zero for large distances ($r \to \infty$) since the interaction has to disappear.

The pair correlation function can be measured in the following way: An arbitrary cell is chosen as a center cell ($r = 0$). The number of cells, $N(r)$, in the area enclosed by the circles with radius, $r - \Delta r$, and r, is determined. The largest circle, r_{max}, is chosen accordingly to the desired maximum distance in the cell-cell interaction. An arbitrary chosen cell should be far enough from the picture's boundary ($> r_{max}$). The procedure is repeated with all possible cells as a center. Then, the procedure is repeated with different pictures.

The normalized pair distribution function was determined from pictures where the distance distribution was expected to be in steady state. The basic results are (Fig.3b): (i) The interaction between two cells is very repulsiv for small distances ($r < 12\mu m$). This repulsion is caused by the steric exclusion - the space occupied by one cell cannot be used by another cell. This steric exclusion was found for the control cells as well as for cells at low calcium. (ii) For large distances ($r > 25\mu m$) the cell-cell interaction is zero. This was found for control cells as well as cells at low calcium. (iii) There is a substantial difference between cells at low calcium and control cells at distances between 12 and 20 μm. Cells at low calcium have an attractive interaction and control cells show a weak repulsion or no interaction.

The nature of the cell-cell interaction is not known. The cell-cell cohesion model is probably less important for granulocytes since these cells attract each other even when there exists no direct cell-cell contact. In principle it is possible that the cell-cell interaction is based on chemical or electrical guiding fields.

Chemotaxis: Every cell can release metabolic molecules since intracellular vesicles are forced to fuse with the plasma membrane in order to expose new receptor molecules. This process is the start of a new machine cycle. This type of cell-cell interaction is important for the aggregation of migrating *Dictyostelium discoideum* amoebae [13].

Galvanotaxis: Every cell is surrounded by an electric field since the ion channels and ion pumps are not homogeneously distributed over the cellular membrane. This electric field can be measured by means of a vibrating electrode [14]. At the leading front there is a net influx current and at the rear end a current out-flux. The electric field measured close to the cell is typically 0.2 V/mm. The electric field lines around a cell can be approximately described by a dipole field. The strongest electric field strength is at the leading front since there the ion channels are concentrated [14]. Thus, the center of the electric dipole is not fixed to the morphological center of the cell but shifted towards the leading front. Galvanotaxis may be important for the aggregation of granulocytes since the electric field sensitivity of one cell is sufficient to registrate the field of another cell.

Granulocytes migrate opposite to the electric field at high calcium concentration [15]. It means one granulocytes could be attracted by the rear end and repulsed by the leading front. In case of two parallel cells one cell would try to move antiparallel to the other one. In average we expect a repulsive cell-cell interaction at high calcium concentration as actually observed. But at low calcium concentration one granulocyte would be attracted by the leading front of another one as actually observed and repulsed by the rear end since the galvanotactic coefficient, k_P, changed sign [12]. In case of two parallel cells one cell would try to move parallel to the other one. Here, we expect an attractive cell-cell interaction as actually observed.

The Cluster Formation

The migrating cells prepared with low calcium, attracted each other and formed clusters out of the uniformly spread cells as can be seen in Fig. 1. The movement of the center of gravity of the whole cluster with its migrating cells was very small. The unification of two clusters was a very seldom event. In the control experiments with high calcium, no cluster formation was observed (1440 and 1100 cells/mm^2). The

cluster formation was a function of the mean cell density, ρ_1. A threshold behavior was observed: At low cell density ($\rho_1 < \rho_{th} = 150-300$ cells/mm^2) the migrating cells did not form clusters. Mainly single migrating cells were observed, but occationally two or three cells stayed together for several minutes. At high cell density ($\rho_1 > \rho_{th}$) the migrating cells formed clusters with a very long lifetime ($> 1h$).

A cluster started at a nucleation center containing two cells. In case of a low cell density, one observed a slow growth process. However, for high cell density a cluster increased its size very fast [12]. For example: $\tau = 6$ min for $\rho_1 = 400$ cells/mm^2 and $\tau = 2.5$ min for $\rho_1 = 1100$ cells/mm^2. The product, $\rho_1 \cdot \tau$, is approximately a constant.

The cluster formation can start with two granulocytes having direct contact of their leading fronts. At this contact the membrane of the leading front showed an enhanced fluctuation. It seemed that one granulocyte was attracted by the leading front of another one. These two 'kissing' cells attracted further cells which tried to get direct contact with the leading fronts of the 'kissing' cells. In large sized clusters the cells are oriented towards the center. The polar order parameter, $\langle cos\varphi \rangle$, was between 0.60 and 0.80 . The cell orientation is singular at the center of the cluster. If a cell mixture of granulocytes and monocytes (small concentration) is used, the nucleation starts preferentially at the monocytes but when the cluster is formed, then, the monocyte is squized out and replaced by a granulocyte.

The cells in a cluster try to move towards the cluster's center. But this movement is hindered since the cluster is already dense packed with cells. Thus, the movement of one cell is successfully if it can take the place of another cell which is squized out. The pressure and the force created by one stopped cell is $\approx 2000Pa$ and 40 nN, respectively [16]. We have a situation where the cells are polar oriented but the center of mass is more or less statistically distributed. Hence, we have a state of a polar nematic liquid crystal: No order in the center of gravity and order in the orientation of the elongated elementary units.

The Mean Field Approximation

We showed in case of low calcium concentration, granulocytes attract each other. The attraction mechanism is based on (i) intrinsic properties of the cell (= automatic controller) and (ii) the extracellular guiding field which is produced by the other cells. The local cell density, n, can be altered by (i) the cell-cell attraction and (ii) the cell diffusion. The temporal change of the cell density, n, is determined by an equation of the form $\partial n/\partial t = gain - loss$. The $gain$ can be approximated with a

mean field approximation. In case of many cells it is difficult to sum up all the pair interaction and to predict the behavior of these cells. Therefore the interaction of *one* with all the *others* can be approximated by introducing a mean guiding field. In the first approximation, the cell orientation is not used explicitly. There the number of attracted cells per unit time is assumed to be proportional to the cell density, ρ, in the vicinity of the cluster and to the number of cells, n, in a cluster. Thus, we have $gain = a_+ \cdot n \cdot \rho$. This model should hold in case of chemotaxis. The second approximation should hold for galvanotaxis: If the guiding field around one cell has a dipolar character then the mean cellular guiding field should be a function of the mean polar orientation of all the other cells, $\langle cos\varphi \rangle$. The dipolar mean field should be zero for random orientation ($\langle cos\varphi \rangle = 0$) and maximum for perfect oriented cells ($\langle cos\varphi \rangle = 1$). For simplicity we assume the mean field is proportional to the mean polar orientation, $\langle cos\varphi \rangle$. The cell-cell interaction is distance dependent and, thus, the dipolar mean field should be a function of the mean cell-cell distance which can be expressed by the mean cell density, ρ. Again for simplicity we assume the mean field is proportional to the cell density, ρ:

$$W = E_0 \cdot \langle cos\varphi \rangle \cdot \frac{\rho}{\rho_{th}} \tag{7}$$

E_0 measures the strength of the mean field. The guiding field, $c_1(field)$, in Eq. 1 has to be replaced by the mean field, W. The angle distribution function can be calculated as shown above (Eq. 3). The equation for calculating the polar order parameter is the self-consistence condition which holds true when $\langle cos\varphi \rangle$ used in the distribution function (Eq. 4) equals the calculated average of $cos\varphi$ (Eq. 5). The dimensionless parameter, β, determines the state of the system.

$$\beta = \frac{k_P}{q_\varphi} \cdot \frac{\rho_1}{\rho_{th}} \cdot E_0 \tag{8}$$

The cell density at the threshold equals ρ_{th}. For $\beta < 1$, the mean order parameter is zero and for $\beta > 1$, the mean polar order is non-zero. Thus, we have a order-disorder transition. The polar mean-field approximation used actually Born and Stumpf [19] to predict a polar nematic liquid crystal built up of molecules having a strong electric dipole moment.

The galvanotactic coefficien, $K_G(= 2k_P/q_\varphi)$ times the strength of the mean field, E_0, is predicted to two at threshold (see Fig. 3a). E_0 may be estimated from the electric field strength around a cell. Typical measured values are 0.2 V/mm [14]. The measured galvanotaxis coefficient is $\approx +4$ mm/V. Thus, the estimated value of $K_G \cdot E_0$ is ≈ 0.8 which is in the right order. Neural crest cells and fibroblasts have even at high Ca^{++}−concentration a positive galvanotaxis coefficient and the absolute

values of the galvanotaxis coefficient are comparable with granulocytes (+6.7 mm/V (neural crest cells)[17] and +3.3 mm/V (fibroblasts)[18]). Thus, it is not surprizing that these cell types form clusters in vivo and in vitro.

The polar order parameter of cells at low calcium in a cluster is very high (0.6 - 0.8). The mean polar field can be determined under the assumption that the cluster formation is based on a galvanotactic response since every granulocyte can be regarded as an electric sensitive detector: One cell is regarded in the field produced by the surrounding cells. The mean polar order parameter of a picked out cell is 0.7 ± 0.1. According to Eq. 5 an electric field of 0.49 V/mm induce an polar order of 0.70 ($K_G = 4mm/V$). This field strength is about twice the field strength of a single cell.

The Outlook

The practical importance of this study lies in the detailed analysis of the experimental conditions. It provides an objective description of cellular cluster formation which may be useful in the embryogenese or organese. The cellular cluster formation is based on the automatic pilot responsible for the directed movement. The objective description may be useful in the definition of cellular dysfunction. For example granulocytes exposed to viruses can lose their ability for directed movement [20]. Furtheremore, the advantage of the technique empolyed is that it is not restricted to granulocytes. It is likely to be used in similar investigations on other cell types or mixtures of different cell types.

Acknowledgement: This work was supported by the "Fondation de France" and the "Deutsche Forschungsgemeinschaft".

References

[1] H. Haken Synergetics (Springer Verlag, Berlin, Heidelberg, New York, 1983)

[2] N. Wiener (1961) Cybernetics: Or control and communication in animal and the machine (MIT Press, Cambridge, 1961)

[3] P.C. Wilkinson Chemotaxis and inflammation (Churchill Livingstone, Edinburgh, London, Melborne, 1982)

[4] J.P. Trinkaus Cells into Organs (Prentice Hall Inc., Englewood Cliffs, New York, 1984)

[5] H. Gruler, in Biological Motion, edited by W. Alt and G. Hoffmann (Springer-Verlag, Heidelberg 1990), pp. 396 - 414

[6] H. Gruler, in Biolocially Inspired Physics, edited by L. Peliti (Plenum Press, New York, London, 1991) pp. 217 - 227

[7] H. Gruler and K. Franke, Z. Naturforsch., 45c, 1241 (1990)

[8] K. Franke, M. Schienbein and H. Gruler, Phys. Rev. E, 49 June (1994)

[9] M. Schienbein and H. Gruler, Bull. Math. Biol., 55, 585 (1993)

[10] B. Rapp, A. de Boisfleury-Chevance and H. Gruler Eur. Biophys. J., 16, 313 (1988)

[11] H. Gruler, in The Neutrophil: Cellular Biochemistry and Physiology edited by M.B. Hallett (CRC Press, Inc. Boca Raton, Florida, 1989), Chap. 3 pp. 64 - 95

[12] H. Gruler and A. de Boisfleury-Chevance, J. Phys. 1 France, 4 July (1994)

[13] B. Alberts, D. Bray, J Lewis, M. Raff, K. Roberts, J.D. Watson, Molecular Biology of The Cell, (Garland Publishing Inc. New York, London, 1983)

[14] R. Nuccitelli, Modern Cell Biology, 2, 451 (1983)

[15] K. Franke and H. Gruler, Eur. Biophys. J., 18, 335 (1990)

[16] H. Gruler, V. Vereycken, and J.-C. Lelièvre, The Linear Motor of Migrating Granulocytes J. Phys. France III, to be published.

[17] H. Gruler and R. Nuccitelli, Cell Motil. Cyto. 19, 121 (1991)

[18] H. Gruler and R. Nuccitelli in Ionic Currents in Development edited by R. Nuccitelli (Alan R. liss, Inc., New York) pp. 337 - 347 (1986)

[19] M. Born and F. Stumpf, Sitzungsberichte der königlich preussischen Akademie der Wissenschaften pp. 1043 - 1060 (1916)

[20] B.D. Bültmann and H. Gruler, J. Cell Biol., 96, 1708 (1983)