Cell Movement and Symmetry of the Cellular Environment

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The movement of micro-organisms was investigated in different cellular environments. The type of movement was described in terms of the symmetry of the cellular environment: (i) Random movement – isotropic symmetry of the environment, (ii) contact guidance – apolar symmetry of the environment and (iii) directed movement as chemotaxis and galvanotaxis – polar symmetry of the environment. To quantitate cell movement it was necessary to parameterize the environment as well as the cell movement by observables.

The random movement was quantitated by the diffusion coefficient. The contact guidance of the nematic type and the contact guidance on a bent surface were quantified by an apolar order parameter. The contact guidance constant for fibroblast on a glass cylinder was 87.5 μm. The directed movement was quantified by a polar order parameter. Dose-response curves were derived and compared for different types of cells: Chemotaxis of granulocytes $K_{CT}^{-1} = 2.2$ mm for 10 μM f-Met-Met-Met, galvanotaxis of granulocytes $K_G^{-1} = 0.2$ V/mm, galvanotaxis of fibroblast $K_G^{-1} = 0.28$ V/mm, galvanotaxis of spermatozoids of bracken fern $K_G^{-1} = 0.024$ V/mm.

Introduction

Directed cellular translocation has been observed in a variety of motile cell types [1] responding to various gradients in their environment. It has been characterized as chemotaxis, necrotaxis, galvanotaxis, haptotaxis, etc. In each of these cases individual cells are observed to exhibit non-random translocation along the direction of the steepest gradient in the magnitude of the external factor. In order to uncover the cellular mechanisms involved in directed locomotion, it is often useful to describe the observed movements mathematically. Information may thus be obtained that is not immediately evident from the data [2, 3].

This concept is not restricted to single cells. It can also be applied to well defined assemblies of cells *e.g.* organs or even whole animals. The concept is also valid for machines as *e.g.* robots, etc. [4].

The cellular locomotion can be approached in the following way: The object of interest can be observed from a birds eye. The cell (organ, animal, robot, etc.) now looks so tiny that it can be represented by a mathematical point. Instead of the need to know all "atomic" coordinates having many degrees of freedom we need to know only a few parameters – the position of the object of observation as a function of time. The experimental recordings of the translocation of our object of interest are our source data. In this concept we do not start from first principles, but it is still possible to look for fundamental laws so that the behaviour of the object of interest can be predicted in a known environment. For example, the chemotactic movement of a cell in a concentration gradient of chemotactic molecules can be predicted if we know the law which holds for this type of movement and the chemotactic constant which quantifies the interaction between the chemotactic molecule and the cell in consideration.

The migration is described in a phenomenological way, without concern for the source of the motion. The aim is to find a small number of unifying laws. These laws can be applied to different cell types, even to organs, whole animals or robots. A typical example is the random walk process of actively migrating cells, of inert particles, or of human beings lost in the fog. These random walk processes are characterized by one unifying law – the diffusion law*. The knowledge of these laws has the advantage of allowing one to predict how a system will behave under certain boundary conditions.

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* Einstein [5] derived the diffusion law for molecules by comparing the molecules with flies in a swarm.

How shall we proceed to find general laws for our objects of observation? The cell collects information from its environment and guides its locomotory machinery. Thus the cellular environment determines the type of locomotion. The symmetry of the cellular environment or the lack thereof is therefore a basic element for the mathematical description of locomotion. The cellular behaviour can be analyzed after we have constructed a mathematical frame work. The experimental data which reflect the response of the cells to their environment are averaged resulting in a parameter quantifying the cellular locomotion. At the end, a general law can be construed when this procedure is repeated for different environmental conditions.

Movement and environment

Chemokinesis

The chemical environment of the object of observation provokes its locomotion. No information is given about whether this locomotion is directed or non-directed locomotion. The change in location along the track, δs, of the moving object in the time interval, δt, is then

$$v_c = \delta s/\delta t. \tag{1}$$

The magnitude of the locomotory activity of the migrating object is described by the mean track velocity, $\langle v_c \rangle$.

The response curve of human granulocytes responding *e.g.* to the chemokinetic molecule f-Met-Leu-Phe (formylmethionylleucylphenylalanine) can be approximated by a hyperbolic curve [6].

$$\langle v_c \rangle = \langle v_{co} \rangle / (1 + K_v^{\text{f-Met-}}/c_{\text{f-Met-}}). \tag{2}$$

$K_v^{\text{f-Met-}}$ is the chemokinetic constant of granulocytes to the chemokinetic molecule f-Met-Leu-Phe, and $\langle v_{co} \rangle$ is the maximum value of the mean track velocity. $K_v/c_{\text{f-Met-}}$ is a dimensionless number which reflects the natural range of cellular sensitivity to the chemokinetic stimulus. If the concentration of the chemokinetic molecules equals K_v the track velocity of the cell is half maximum. The chemokinetic constant, K_v, for f-Met-Leu-Phe is 0.3 nM [6]. K_v has about the same value as the high affinity receptor to f-Met-Leu-Phe (0.53 nM [7]). This indicates that the cellular response to the external factor is already determined by receptors incorporated in the membrane. The cellular chemokinetic activity treated in analogy to the kinetics of enzyme reactions, is discussed elsewhere [8].

Isotropic random walk

The locomotion of a cell is isotropic if a cell is exposed to an environment having an isotropic symmetry. At every position and at every orientation the cell obtains the same information. The result is that the cell exhibits a random walk movement in any direction. This means the migrating cell moves in a random fashion around an arbitrarly chosen starting position. The average of the displacements, $\langle x \rangle$ and $\langle y \rangle$, in a given time, t, is zero due to the symmetry of the cellular environment. This means that the cell migrates all the time, but the center of gravity of all the migrating cells does not change with time.

The mean square displacement is a non-zero value which can be used to quantify non-directed locomotion [6, 9–12]. Einstein [5] and von Smoluchowski [13] were able to quantify the random walk.

$$\tfrac{1}{2}(\langle x^2 \rangle + \langle y^2 \rangle) = 2\,D\,t. \tag{3}$$

The diffusion coefficient D characterizes the intensity of the random walk. As early as 1913 Przibram [14] showed that the random locomotion of protozoa can be described by the Einstein-Smoluchowski theory.

Fürth [15] introduced the correlated walker. He idealized the trajectories of the migrating cells by a sequence of straight-line steps and obtained for this correlated walk in one dimension the so called Langevin [16] equation which quantifies the random walk or diffusion process much better than the Einstein-Smoluchowski theory

$$\tfrac{1}{2}(\langle x^2 \rangle + \langle y^2 \rangle) = 2\,D\,\{t - \tau(1 - e^{-t/\tau})\} \tag{4}$$

with

$$D = \tfrac{1}{2}\langle v_c^2 \rangle\,\tau/n. \tag{5}$$

τ is the time interval between two successive changes in the moving direction and n is the dimensionality of the movement. Fürth [15] applied his theory to the random walk of infusoria and showed that these cells are correlated walkers. Other cell types are also correlated walkers: bracken spermatozoids of fern [17], fibroblasts [18], sponge cells [19], granulocytes [12, 20, 21], and slime-mold amoebae [22].

Hall [22] idealized the trajectories of migrating micro-organisms by a zig-zag line and extended Fürth's work to a correlated walker in two dimen-

sions. The result is an equation with the structure of the Langevin equation*

$$\tfrac{1}{2}(\langle x^2\rangle + \langle y^2\rangle) = 2\,D\,t - B(1 - \langle\cos\varnothing\rangle^{t/\tau}) \qquad (6)$$

with

$$D = \tfrac{1}{2}\langle v_c^2\rangle\,\tau\,(1 + \langle\cos\varnothing\rangle)\,(1 - \langle\cos\varnothing\rangle)^{-1}n^{-1} \qquad (7)$$

$$B = \langle v_c^2\rangle\,\tau^2\,\langle\cos\varnothing\rangle\,(1 - \langle\cos\varnothing\rangle)^{-2}/n. \qquad (8)$$

$\varnothing$ is the angle through which the cells turn from step to step. Hall applied his theory to the random movement of slime-mold amoebae and found good agreement between the experiment and the correlated walk model. The right function of the mean square displacement *vs.* time is found and in addition the magnitude of the diffusion coefficient is predicted. Hall's model could also be applied to other cell types as long as the path can be approximated by a zig-zag line.

We have enough experimental material of migrating human granulocytes to show that the correlated walk model of Hall also holds for this cell type: The mean square displacement of human granulocytes is shown in Fig. 1 as a function of time. The dots represent actual measurements obtained directly from the paths. If we fit the Langevin equation (Eqn. (4)) to the experimental data then the diffusion coefficient ($D = 218\ \mu m^2\ min^{-1}$) and the characteristic time ($\tau = 50$ s) are fitting parameters (thick line in Fig. 1). If we use Hall's model the diffusion coefficient is not a fitting parameter. It can be obtained from the velocity of the cells, from the time interval between two successive changes in direction, and from the angular change after the straight line path. The values of the mean square track velocity, $\langle v_c^2\rangle$, the characteristic time, τ, and the average of cos $\varnothing$ were determined in different ways from the trajectories as shown for example in Ref. [12]. The track velocity distribution function is used to calculate the mean square track velocity, $\langle v_c^2\rangle$ ($= 540\ (\mu m/min)^2$). The characteristic time, τ ($= 0.7$ min), and the average of cos $\varnothing$ ($= 0.6$) were determined from the time dependent angular distribution function $f(\varnothing; t)$; an example is shown in Ref. [9] and [12]. The result is the thin line in Fig. 1. The course of the points is very well described but the predicted diffusion coefficient is too small ($D = 189\ \mu m^2\ min^{-1}$). The small discrepancy between theoretical and experimental results is that the small memory time ($\tau = 1.2$ min) is not yet considered in the theoretical approach. It was Othmer *et al.* [23] who enlarged the theory and found good agreement between theory and the experiments.

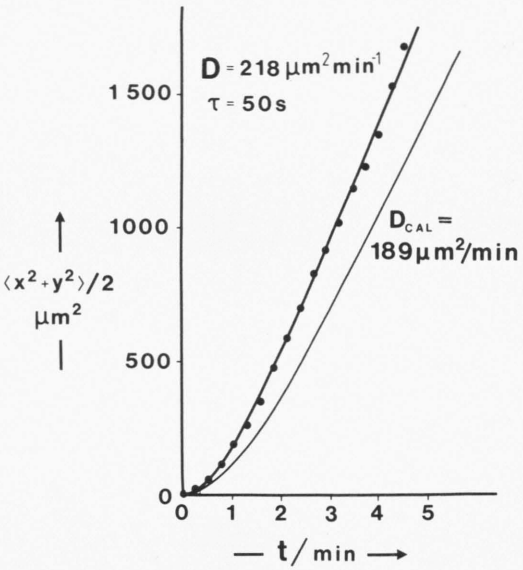

Fig. 1. Mean square displacement of migrating granulocytes as a function of time: granulocytes (actual measurements, thick line predicted by Eqn. (4) and thin line predicted by Eqn. (6), (7), and (8)).

The random movement of human monocytes can be approximated by a zig-zag line, but Hall's theory fails here since, as we have shown [24] this cell type has a long directional memory time. The random movement of lymphocytes also cannot be described by a correlated walk model since the trajectories of these cells can not be simply approximated by a zig-zag line [24]. Othmer's theory [23] is not yet applied to migrating monocytes.

Anisotropic random walk – contact guidance

The cell migration is anisotropic if the cellular environment is anisotropic. The prototype of this type of movement is the contact guidance.

The contact guidance is the ability of some cell types (granulocytes, fibroblasts, tumor cells, etc.) to orient their locomotion along physical structures as for example oriented macromolecules, thin lines, bent surfaces, etc. [25–29]. At every position and at every orientation the cell obtains the same chemical information, but since the cellular environment is

* Hall's calculations were simplified: a constant instead of a variable step length was used here.

anisotropic, the cells obtain different structural information at every position and at every orientation. The result is an anisotropic movement.

The contact guidance of the moving cells is much more complex than the isotropic random walk. It can be subdivided in at least three categories [9, 30]*: (i) The nematic type of contact guidance occurs when the cell size is large in relation to the characteristic length of the anisotropic surface. For example, a granulocyte is crawling on a flat piece of glass coated with well oriented collagen fibers. The characteristic length of the substrate is then the distance between two macromolecules. (ii) The smectic type of contact guidance occurs when the cell size is small in relation to the characteristic length of the substrate. For example, a granulocyte is crawling on a ground plate of a Neubauer counting chamber where the distance between two neighbouring lines is much larger than the size of the cell. (iii) The cells can guide their locomotion on bent surfaces. For example a fibroblast tries to orient its long axis in the direction of the lowest curvature.

a) Nematic contact guidance

In the nematic contact guidance, the cell obtains the information which depends only on the orientation of the cell with respect to the physical structure of the substrate. Therefore the nematic contact guidance is characterized by an angle-dependent random walk process. The angle-dependent diffusion coefficients can be described by an ellipse.** The deviation from the circle defines an apolar order parameter, $\langle P_2 \rangle$, which quantifies the nematic contact guidance [9, 30].

$$\langle P_2 \rangle = (D_x - D_y)/(D_x + D_y). \tag{9}$$

This apolar order parameter is zero when the diffusion coefficient, D_x, measured parallel to the lines equals the diffusion coefficient, D_y, measured perpendicular to the lines. The apolar order parameter equals one if the diffusion coefficient perpendicular to the lines is zero. In this case the random walk is a one-dimensional process parallel to the lines. The movement can be compared with a "twoway" traffic along the lines. Correspondingly, the directed locomotion would be a "oneway" traffic. The apolar order parameter is minus one if the diffusion coefficient parallel to the lines is zero and nonzero if it is perpendicular to the lines. In this case the diffusion process is a "twoway" traffic perpendicular to the lines. Typical values of the apolar order parameter of granulocytes migrating on an optical grid, a scratched aluminium surface, a stretched polyethylene foil, etc. are +0.3 [9, 30].

The trajectories of migrating granulocytes is still a zig-zag line. The question is can Hall's model still be applied to this anisotropic movement? The answer is yes! The apolar surrounding as for example oriented macromolecules, etc. induces an angle-dependent track velocity but the angular change in moving direction is less sensitive to such an environment (Matthes and Gruler [30]). The result is that the mean length of the straight line segments of the zig-zag line is angle-dependent.

Can we now make predictions how cells will behave in a certain environment? The answer is still no! The dose-response curve of the nematic contact guidance is not known. The problem is the environment, which is hard to quantify. Successful progress is made by describing the environment by the anisotropic surface tension [30]. But the problem has not been solved as yet.

b) Smectic contact guidance

In this case the cell obtains from the substrate information dependent on the position and on the orientation of the cell. The smectic contact guidance can be characterized by an angle-dependent and position-dependent random walk process. The cellular response to the structure can partially be quantitated by the position-dependent cellular density which for a flat surface is a constant. The deviation from this constant cell density defines a density order parameter, $\langle P_{1d} \rangle$, which quantifies the smectic contact guidance [9, 30]. When the cells do not recognize the undulations of the surface the density order parameter is zero. If, on the contrary, the cells are attracted by lines in the surface and concentrated on them, the density order parameter is one. If the cells are repulsed from the lines and concentrated in the middle between two lines the density order parame-

* Liquid crystal phases and the different types of contact guidance are closely related in the phenomenological description. The nematic type of contact guidance is similar to the nematic liquid crystal, and the smectic type of contact guidance is similar to the smectic liquid crystal.

** The same type of movement is obtained if one allows molecules to diffuse in a nematic liquid crystal [31].

ter becomes minus one. For example, the density order parameter of granulocytes exposed to a Neubauer chamber where the distance between two lines is 50 μm, is +0.2 [30].

This type of contact guidance is not so well understood as the nematic contact guidance. No dose-response curve is available.

c) Contact guidance on a bent surface

P. Weiss [32] observed that fibroblasts orient themselves on bent surfaces. For example, fibroblasts exposed to the outer surface of a glass cylinder orient in the mean their long axis parallel to the axis of the cylinder. In this case the cellular environment can be quantified by the curvature of the surface and the cellular response can be quantitated by the apolar order parameter, $\langle P_2 \rangle$, which can also be determined by the mean of cos 2 ∅, so that a dose-response curve can be obtained. P. Weiss [32], Abercrombie [33] showed that fibroblasts exposed to a thin cylinder with its strong curvature are very well oriented. However, cells exposed to a thick cylinder with its small curvature show nearly no orientation. The curvature, K $(=R_0^{-1})$, of the cylinder with a radius, R_0, may be considered the "dose" for the cellular reaction. The predicted dose-response curve is* [34]

$$\langle P_2 \rangle = I_1((K_{CG}\, K)^2)/(I_0((K_{CG}\, K)^2). \tag{10}$$

I_1 and I_0 are hyperbolic Bessel functions, and the constant, K_{CG}, quantifies the interaction of the cells with the bent substrate. The method is applied to fibroblast which are oriented parallel to the long axis of the cylinder ($K_{CG} = 87.5$ μm [34]). For this case the apolar order parameter is 0.446. The cells show only a small tendency to orient themselves on a cylinder with a radius of 220 μm where the expected apolar order parameter is 0.15, and one expects an apolar order parameter of 0.85 if the fibroblasts are exposed to a cylinder with a radius of 65 μm. If the dose-response curve which quantifies the cellular reaction to its environment is known the cellular behaviour can be predicted. Up to now no theory is available for predicting the contact guidance constant K_{CG} from first principles.

* The procedure used to obtain a dose-response curve is explained in the next chapter.

Directed locomotion

The directed locomotion of cells is obtained when the cellular environment has a polar symmetry. For example, a cell exposed to an electric field, a concentration gradient of chemotactic molecules, etc. shows a directed locomotion which is referred to as galvanotaxis, chemotaxis, etc. In general, the cells collect information from their environment and guide their movement in such a way that the cells drift parallel to the direction of the polar field.

The mean displacement, $\langle x \rangle$, quantifies the directed locomotion if the direction of the polar field is parallel to the x-axis.

$$\langle x(t) \rangle = \langle v_{II} \rangle\, t. \tag{11}$$

The mean velocity parallel to the direction of the polar field, $\langle v_{II} \rangle$, is also referred as drift velocity. The mean displacement, $\langle y \rangle$, of the cells measured perpendicular to the direction of the polar field is zero since the movement in this direction is random.

The center of gravity of many cells changes in time and drifts parallel to the direction of the polar field as described by Eqn. (11). Therefore the important biological function of chemotaxis, galvanotaxis, etc. is described by this simple equation. The same type of movement is obtained if an object falls, driven by gravity or another constant force, in a viscous liquid. One can say that the constant polar field acts on the cells as a constant force. The drift velocity, $\langle v_{II} \rangle$, in the case of the falling object, is given by the applied force divided by the friction coefficient. When the object is spherical, the friction coefficient can be expressed by Stokes' law, so that the drift velocity depends only on the mass and the radius of the object and on the viscosity of the liquid. The question is, can we find a law comparable to Stokes' law which would enable us to predict the movement of the cells? The answer is yes! The procedure for finding the law is fairly complex, therefore we shall explain it in three steps:

Step 1: Polar order parameter, drift- and track velocity

Let us start with a well-known mathematical expression: The projection of the track velocity, $v_c(t)$, at the time t upon the direction of the polar field ($v_{II}(t)$) is (see Fig. 2)

$$v_{II}(t) = v_c(t) \cos \varnothing\,(t). \tag{12}$$

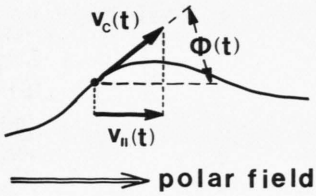

Fig. 2. The relation at a fixed time, t, between the track velocity, $v_c(t)$, the drift velocity, $v_{II}(t)$, and the angle of migration, $\varnothing(t)$.

The angle of migration, $\varnothing(t)$, as well as the track velocity, $v_c(t)$, change their values with time. The average of this equation leads to the mean drift velocity. The average values of v_{II}, v_c and cos $\varnothing$ are related as

$$\langle v_{II} \rangle = \langle v_c \rangle \langle \cos \varnothing \rangle \quad (13)$$

if the time dependence of the track velocity is *not correlated* with the time dependence of the angle of migration. This is true at least for granulocytes [35, 36] and monocytes [24] migrating in a necrotactic gradient and for fibroblasts [37] and for neural crest cells [38] migrating in an electric field. The chemokinetic activity is thus independent of the cellular decision activity. In further discussion, we can restrict us to the average value of cos $\varnothing$* the so-called polar order parameter $\langle P_1 \rangle$.

The polar order parameter is the quantity which characterizes the directed locomotion: It is zero for a non-directed locomotion. The polar order parameter is +1 if the cells migrate parallel to and in the direction of the polar field. The polar order parameter is −1 if the cells locomote parallel to but in the opposite direction from the polar field.

Let us look at a few examples: The polar order parameter for human granulocytes determined on the basis of experiments in a Zigmond chamber with 10 nM/mm f-Met-Leu-Phe and 5 nM f-Met-Leu-Phe is -0.85 ± 0.05 [8, 36]. The polar order parameter is negative since the direction of the polar field is defined by the diffusing chemotactic molecules and the granulocytes drift in the opposite direction with respect to the diffusing chemotactic molecules. The polar order parameter for human granulocytes and monocytes in a necrotactic assay, in which an erythrocyte is lysed by a laser beam, is -0.85 ± 0.05 [35]. The polar order parameter of human granulocytes exposed to an electric field of 0.4 V/mm is -0.65 ± 0.1 [38, 39]. The polar order parameter for human granulocytes exposed close to a clump of bacteria is between −0.8 and −0.9 [1].

The polar order parameter expresses a cell's ability to recognize the polar field. $\langle P_1 \rangle$ will be low when the polar field strength is very low and it will be close to one if the polar field strength is high. How the polar order parameter depends on the strength of the polar field will be derived in the second step.

Step 2: Distribution function and generating function

The polar order parameter permits to distinguish between directed and non-directed locomotion and is therefore of fundamental interest. New insights into cellular locomotion can be obtained when the angular distribution function, $f_p(\varnothing)$, of the migrating cells, as shown in Fig. 3, is investigated instead of the average of cos $\varnothing$. It is clear that a large amount of information is lost during this averaging procedure.

The angular distribution function of the directed locomotion is a bellshaped curve with its maximum plotted in a direction opposite to that of the applied polar field. The angular distribution function can be approximated by a gaussian curve. However, the gaussian has not be same symmetry as the experimental assay and therefore it is not a good description [2].

The following general procedure can be applied to get a good description of the angular distribution function: The distribution function is expressed by its

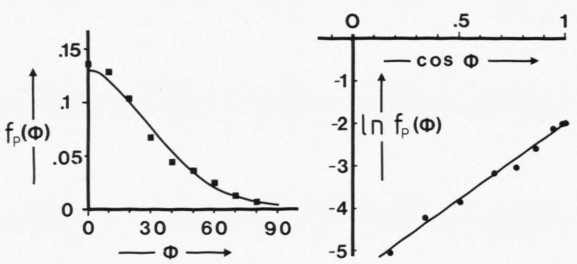

Fig. 3. Polar distribution function of migrating granulocytes in a concentration gradient of chemotactic molecules (10 nM/mm and 5 nM f-Met-Leu-Phe). a) $f_p(\varnothing)$ *vs.* $\varnothing$ and b) f_p *vs.* cos $\varnothing$ where a straight line is fitted to the data.

* The polar order parameter [2, 6, 9, 12, 17, 30, 31, 36], $\langle P_1 \rangle$, as the mean of cos $\varnothing$ is identical to the chemotropism index, $\langle v_{II} \rangle / \langle v_c \rangle$, and the McCutcheon index (ratio between travel parallel to the direction of the polar field and the actual distance travelled along the trajectory).

generating function, $V(\varnothing)$, without loss of information [41].

$$f_p(\varnothing) = e^{V(\varnothing)}. \tag{14}$$

Then the generating function is expressed by a Fourier series. At the end of the procedure the symmetry of the system is applied to the Fourier series. The angular distribution function characterizing directed movements is [2, 41]

$$f_p(\varnothing) = e^{a + b\cos\varnothing + c\cos 2\varnothing + \ldots}. \tag{15}$$

The measured angular distribution function is very well approximated by two terms of the Fourier series (Eqn. (15))* as can be seen in Fig. 3b (see also *e.g.* Ref. [2]).

The polar order parameter, as the average of cos $\varnothing$, can be calculated from Eqn. (14). The final result [2, 9, 42] is the ratio of two hyperbolic Bessel functions [43]

$$\langle P_1 \rangle = I_1(b)/I_0(b). \tag{16}$$

This equation holds for cells migrating in a two-dimensional space (for example, cells crawling on a surface, swimming in a narrow gap, etc.). If the cells swim in a three dimensional space, Eqn. (16) has to be replaced by the Langevin function.

$$\langle P_1 \rangle = \coth b - 1/b. \tag{17}$$

In the last step we have to find a relation between the coefficient b and the strength of the applied polar field.

Step 3: Thermodynamic arguments

Our aim is to find an analytic expression for the dependence of coefficient b on the polar field strength. To solve this problem it is necessary to have a theoretical framework: a thermodynamic one will be used here.

It is well accepted that the first step in chemotaxis is based on a chemical reaction of the membrane-bound receptor with the chemotactic molecule followed by a sequence of biochemical and biophysical reactions. The Gibbs free energy [44] has to be considered in the case of a reversible chemical reaction. The thermodynamic force [45] has to be considered in the case of an irreversible binding. In both cases the important quantity is the logarithm of the concentration of the chemotactic molecules. Therefore one expects

$$b = (K_{CT} \operatorname{grad} \ln c)^h. \tag{18}$$

The chemotactic constant, K_{CT}, defines how sensitive the cells are to the concentration gradient of the chemotactic molecules. If the chemical reaction is cooperative then the cooperativity coefficient h is not equal to one. In the case that the concentration depends on only *one* coordinate the polar field reads $c^{-1}\, dc/dx$. The polar field is proportional to dc/dx, as expected; in addition, it is inversely proportional to the concentration.

The galvanotactic response can be described in a similar way.

$$b = (K_G \operatorname{grad} V)^h. \tag{19}$$

The galvanotactic constant, K_G, describes how sensitive the cells are to electric fields E ($= -\operatorname{grad} V$) or electrical potentials, V. The polar field in galvanotaxis is the applied electric field strength.

Is there any proof for Eqn. (18) and (19)? Brokaw [46] investigated the chemotaxis of bracken spermatozoids of fern over a wide range of the concentration gradient and he found a linear relation between the electric field strength and the gradient of the logarithm of the concentration of the chemotactic molecules as predicted by Eqn. (18) and (19). The cellular response induced by an electric field strength is equivalent to the cellular response induced by a polar chemotactic field. In addition one can show by using Brokaw's [46] data (see Fig. 4) that the cooperativity coefficient for the galvanotactic response of spermatozoids is one.

The calculation of the polar order parameter as a function of the strength of the polar field is based on the measured angle-distribution function. Therefore, *any* directed movement of *any* cell can be analyzed. In addition the beauty of this mathematical treatment is that any type of orienting gradient or cellular response can be directly plotted on the same graph for comparison if the applied polar field strength is measured in natural units (= dimensionless numbers) [2]. The following examples are presented in Fig. 4:

(i) Galvanotaxis of granulocytes: Rapp *et al.* [39] measured the galvanotactic response of human granulocytes (+ in Fig. 4). A cooperativity coefficient of one gives the best fit to the data indicating that the galvanotactic response of granulocytes presumably re-

* Only the unknown coefficient, b, has to be determined; the coefficient, a, is already determined by the calibration procedure, $\int f_p(\varnothing)\, d\varnothing = 1$.

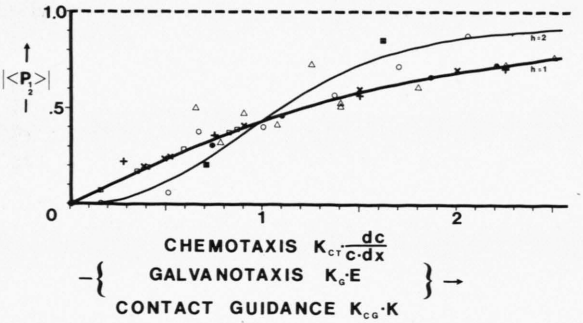

Fig. 4. Polar order parameter, $\langle P_1 \rangle$, is shown as a function of the strength of the polar chemotactic field, $c^{-1}\, dc/dx$, and of the electric field strength, E. The polar fields are measured in units of the inverse of the chemotactic and galvanotactic constant, K_{CT}, and K_G, respectively. Granulocytes exposed to f-Met-Met-Met (data from Ref. [48]): ● $c = 10$ μM, dc/dx = variable, $K_{CT} = 2.2$ mm; × $c^{-1}\, dc/dx = 0.82$ and □ $c^{-1}\, dc/dx = 0.5$ where c and K_{CT} are variable; + granulocytes exposed to electric fields E with $K_G^{-1} = -0.2$ V/mm (data from Ref. [40]); ○ fibroblasts exposed to electric fields with $K_G^{-1} = 0.3$ V/mm and $h = 2$ (data from Ref. [2] and [44]); △ bracken spermatozoids exposed to electric fields with $K_G^{-1} = 0.024$ V/mm (data from Ref. [47]). The apolar order parameter, $\langle P_2 \rangle$, is also shown as a function of the curvature, K. ■ fibroplasts exposed to glass cylinders with $K_{CG} = 87.5$ μm and $h = 2$ (data from Ref. [31]). Theoretical predictions from Eqn. (7), (13), (14), and (15): thick line ($h = 1$) for a non-cooperative process and thin line ($h = 2$) for a cooperative process.

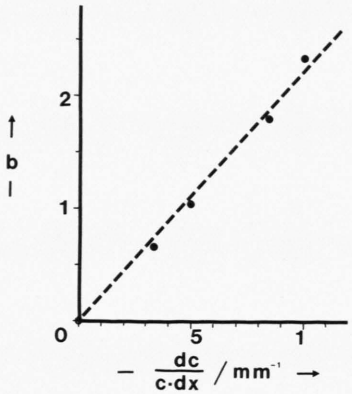

Fig. 5. The coefficient, b, as function of the polar chemotactic field, $c^{-1}\, dc/dx$. In this experiment the concentration gradient, dc/dx, of the chemotactic molecules was varied but the concentration, c, of the chemotactic molecules was the same (data from Ref. [48]).

flects a non-cooperative process. The galvanotactic constant, K_G^{-1}, is -0.2 V/mm. Granulocytes drift towards the anode so that the polar order parameter is negative. The galvanotactic constant can be predicted by a molecular model which will be published somewhere else [8].

(ii) Chemotaxis of granulocytes: Zigmond [47] investigated the chemotactic response of granulocytes. The theoretically derived equations were fitted to her published data. The percentage of orientation which Zigmond introduced to characterize the directed locomotion were used to calculate the polar order parameter on the basis of Eqn. (15). The coefficient b as a function of $c^{-1}\, dc/dx$ (data: Fig. 7 of Ref. [47]) is a straight line (Fig. 5). The cooperativity coefficient is obviously 1. However there is a difficulty: The chemotactic constant is a function of the concentration of the chemotactic molecule.* The result is a bell-shaped curve as shown in Fig. 6 where K_{CT} is plotted *vs.* log c for all the available data (Fig. 5, 7 and 8 of Ref. [47]). Obviously the chemotactic response of granulocytes is much more complex than the response of bracken spermatozoids of fern.

Bacteria and spermatozoids measure the concentration of chemotactic molecules at different times to obtain the concentration gradient. In this case the electric field strength and the gradient of the logarithm of the chemotactic molecules induce the same physiological reaction. But if the cell measures at one time the concentration of the chemotactic molecules at two places and if the cell compares the

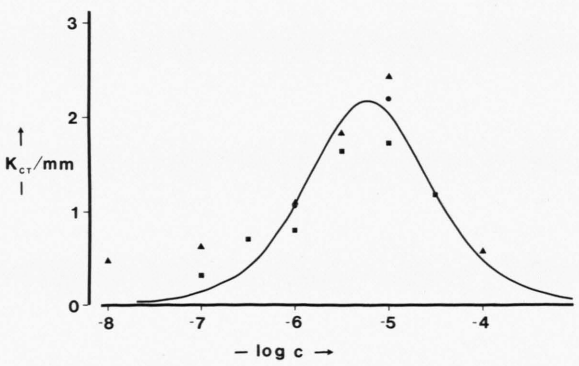

Fig. 6. The chemotactic constant, K_{CT}, as a function of the logarithm of the concentration of the chemotactic molecules f-Met-Met-Met (data from Ref. [48]).

* In a previous paper [2] we concluded that the chemotaxis of granulocytes is a positive cooperative phenomenon since we did not know that the galvanotactic constant is not a constant.

two measured results than the chemotactic constant is a function of the mean concentration of the chemotactic molecules, c_o, as model calculation has shown [8].

$$K_{CT} = A\,(c_0/K)\,(1 + c_o/K)^{-2}. \quad (20)$$

This model predicts that the chemotactic constant has a maximum when the mean concentration of the chemotactic molecule equals the equilibrium binding constant, K, of the chemotactic molecule to the receptor. The theoretically predicted curve (Eqn. (20)) is shown in Fig. 6 where one of the two fitting parameters is the equilibrium binding constant of f-Met-Met-Met to the receptor ($K = 6.3$ μM). The theoretically predicted curve fits quite well to the experimental data. The discrepancy at small mean concentration may be due to the fact that there exists a high and a low affinity receptor but in the theoretical treatment only one affinity site was taken into account.

(iii) Galvanotaxis of fibroblasts: Nuccitelli and Erickson [48] measured the galvanotactic response of fibroblast. Gruler and Nuccitelli [2] analyzed their data and obtained $K_G^{-1} = 0.28$ V/mm with a cooperativity coefficient of two. Fibroblasts drift towards the cathode so that the polar order parameter is positive.

(iv) Galvanotaxis of bracken spermatozoids of fern: Brokaw [46] measured the directed movement and the analysis leads to $K_G^{-1} = 0.0238$ V/mm and a cooperativity coefficient of one. Here it is necessary to remember that Brokaw fitted his data to the Langevin function. But the cells can only swim in two dimensions (= narrow gap) so that the ratio of the Bessel functions has to be applied.

(v) Contact guidance of fibroblasts: Abercrombie [33] measured the contact guidance of fibroblasts exposed to glass cylinders of different thicknesses. We analyzed these data and obtained a contact guidance constant, K_{CG}, of 87.5 μm and a cooperativity coefficient of two.

(vi) Growth of Fungal Hyphe in an electric field: Gow and McGillivray [49] measured the cellular grow of Fungal Hyphe in an electric field. We analyzed the data [50]. The following results were obtained: (a) The cells grew towards the anode. (b) The cellular response was linear for small sized cells and weak electric field strengths ($K_{GROW}^{-1} = -2.0$ V/mm for $l = 10$ μm and -0.2 V/mm for $l = 100$ μm). (c) The galvanotropic response was non-linear with a negative cooperativity for E^2l greater than 4.4 V^2cm^{-1}. (d) The galvanotropic response of longer hyphae was also bidirectional with a linear response ($K_2^{-1} = -1.67$ V/mm; $E^2l > 4.4$ V^2cm^{-1}).

In summary: We can predict how a cell will behave in a concentration gradient of chemotactic molecules or in an electric field if we know the chemotactic or the galvanotactic constant, and the cooperativity coefficient.

Concluding remarks

We demonstrated that a detailed analysis of cell movement lead to new insights: The phenomenological fundamental laws of the cell movement were derived so that it is possible to predict cellular movements if the cellular environment is known. We showed that the symmetry of the environment of the cell is crucial as in solid state physics or crystallography.

Every phenomenological law contains coefficients which are determined by fitting experimental data to a theoretically predicted curve. Such coefficients can be very accurate since their values are based on many data points. Naturally, the phenomenological coefficients may be changed by experimental conditions and may thus be taken into account for questions like what are the proteins essential for the galvanotaxis of granulocytes? So far, nothing is known! But if, for example, the directed movement could be demonstrated to be pH-dependent and if the galvanotactic constant could be shown to have an isoelectric point as we have demonstrated before [38, 39] then the protein candida essential for galvanotaxis will be restricted to only a few which have the right isoelectric point. In the case of granulocytes, the membrane-bound G-protein is obviously the protein responsible for galvanotaxis since the isoelectric point of the galvanotactic response and of the G-protein is pH 5.7. To pose another question: is the directed movement a function of the density of the mikrotubuli or of the microfilaments? To answer this question, the directed movement has to be measured as a function of the concentration of colchicine or cytochalasin B. As it turned out [51] the polar order parameter is slightly decreased at cells with disrupted microtubuli since the turn angle of the cells is more random but the mean track velocity is unaffected by the disrupted microtubuli [51]. The density of the microfilaments, however, has no effect on the polar order parameter. But the mean track velocity is strongly influenced by the density of well working microfilaments [51].

The knowledge of the phenomenological coefficients allows us to make physiolocially important predictions. For example the random walk behaviour of migrating cells can be regarded as the mode searching for chemical or electrical gradients (= searching strategy). The random walk as described by the Langevin equation, is quantified by the diffusion coefficient. How the searching mode is disturbed by colchicine can by assessed by the diffusion coefficient. When a colchicine treated cell has found a chemical or electrical gradient then the directed movement will be deduced. The situation discussed here for colchicine, holds also for other drug molecules.

As has been shown [51] the behaviour of the moving cell can be simulated by a micro-computer in such a way that it is possible to simulate the *in vivo* situation in order to be able to find an optimal drug concentration for a given environmental situation.

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Appendix

Symmetry operations

The type of cell movement is a function of the type of environment. To quanitate cell movement, it is necessary to parameterize the environment as well as the cell movement by observables, N, where N stands for an angle distribution function or a cell density distribution function, etc. These observables are described in an appropriate coordinate system. If the coordinate system is changed in such a way that the physical state of the system is the same as in the previous coordinate system then one has found a symmetry element of the system. In the following we will show the symmetry porperties for different experimental systems.

1) Flat surface (homogeneous and isotropic cellular environment):

$$N(r,\varnothing) = N(r+\delta r,\varnothing) \text{ with } \delta r \text{ arbitrary}$$
$$N(r,\varnothing) = N(r,\varnothing+\delta\,\varnothing) \text{ with } \delta\,\varnothing \text{ arbitrary.}$$

These symmetry properties lead to a random walk.

2) Periodic surface with an apolar symmetry:

(Surface is in the xy-plane, and the periodic lines are in the y-direction. The origin of the coordinate system goes through one line.)

$$N(x,y) = N(-x,y)$$
$$N(x,y) = N(x,-y)$$
$$N(x,y) = N(x,y+\delta y) \quad \text{with } \delta y \text{ arbitrary}$$
$$N(x,y) = N(x \pm k\ L, y) \quad \text{with } k \text{ a natural number and } L \text{ the periodic length.}$$

These symmetry properties lead to an anisotropic random walk. One obtains the smectic contact guidance when the cell registrates all four symmetry elements. The nematic type of contact guidance is obtained when the cell recognizes only the first three symmetry properties. (If the cell is too large it can not recognize the last symmetry property.)

3) Surface of a cylinder:

(The symmetry axis (long axis) of the cylinder goes through the origin and points in the z-direction.)

$$N(R_0,\Theta,z) = N(R_0,\Theta+\delta\Theta,z) \text{ with } \delta\Theta \text{ arbitrary}$$
$$N(R_0,\Theta,z) = N(R_0,\Theta,z+\delta z) \text{ with } \delta z \text{ arbitrary}$$
$$N(R_0,\Theta,z) = N(R_0,-\Theta,z)$$
$$N(R_0,\Theta,z) = N(R_0,\Theta,-z).$$

These symmetry properties lead to an anisotropic random walk – the contact guidance on a bent surface.

4) Constant concentration gradient:

(Polar symmetry axis goes through the origin of the coordinate system and points in the x-direction.)

$$N(x,y,z) = N(x,-y,z)$$
$$N(x,y,z) = N(x,y,-z).$$

These symmetry properties lead to the directed movement – the chemotaxis.

5) Homogeneous electric field:

(Polar symmetry axis goes through the origin of the coordinate system and points in the x-direction.)

$$N(x,y,z) = N(x,-y,z)$$
$$N(x,y,z) = N(x,y,-z).$$

These symmetry properties lead to the directed movement – the galvanotaxis.

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