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Analysis of cell locomotion

Contact guidance of human polymorphonuclear leukocytes

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Abstract. The methods of statistical physics have been applied to the analysis of cell movement. Human polymorphonuclear leukocytes were exposed to different surfaces possessing parallel oriented physical stuctures (scratched glass surface, machine drilled aluminium surface, optical grid and stretched polyethylene foil) and cell migration was observed using time-lapse photography.

We demonstrate that in cell migration along physical structures, referred to as contact guidance, two subgroups can be distinguished: *1)* The nematic type where the cell size is large in relation to the grid distance of the undulate surface. *2)* The smectic type where the cell size is small in relation to the grid distance of the substrate.

Nematic contact guidance is characterized by an anisotropic random walk. In all substrates investigated the diffusion process parallel to the lines was faster than the diffusion process perpendicular to them. The angular dependent diffusion coefficient was described by an ellipse. Deviation from a circle defined an apolar order parameter, whose value was about 0.3. The amount of information which the cells collected from, the undulate surface was very low, between 0.1 and 0.2 bits. We demonstrate that cells do not recognize all the details of their surroundings and that their migration can be compared to the "groping around" of a short sighted man. The blurred environment can be described by a mean field whose strength is proportional to the aploar order parameter. It is argued that the anisotropic surface tension is the basic source for nematic contact guidance.

Smectic contact guidance is characterized by an anisotropic random walk and is quantified by a density order paramter which is 0.28 in the case of the scratched glass surface of a Neubauer counting chamber. The information which the cells collect from their environment is very low (0.03 bits). The lines seen by the cell can be described by a mean field whose strength is proportional to the density oder parameter.

Finally, we demonstrate that the locomotion of granulocytes is governed by an internal clock and internal programs. After migrating for a certain time (32 s) in a particular direction, a new direction of locomotion is determined by an internal program. The cell decides basically between left or right, thereby preferring a turn angle such that the cell migrates either parallel or perpendicular to the lines. The angles are nearly equally probable but the cell moves, in the case of nematic guidance, with different velocities in the + or − direction. The cell also has directional memories with characteristic times of 32 s and greater than 100 s.

Key words: Cell locomotion, contact guidance, leukocytes

I. Introduction

The ability of some cells or primitive organisms to orient their movement along a chemical gradient seems to be a general phenomenon which is referred to as chemotaxis (Wilkinson 1983). Furthermore, some cell types orient their movement along physical structures. This phenomenon, referred to as contact guidance, was first described by Harrison (1912), who had observed that moving fibroblasts orient their movement on the threads of a spider web. Several workers (Weiss 1959, 1961; Weiss and Garber 1952; Elsdale and Bard 1972; Dunn and Ebendal 1978; Dunn and Heath 1976; Ebendal 1977 ; Haston et al. 1982, 1983; Lackie and Wilkinson 1894; Wilkinson and Lackie 1983) have noted that fibroblasts orient their movement along scratches on a glass plate or along collagen fibres. Haston et al. (1983), Lackie and Wilkinson (1984) and Wilkinson and Lackie (1983) demonstrated that polymorphonuclear leukocytes have the ability to orient themselves along dried collagen fibres coated on glass.

In this article, we present a detailed analysis of cell locomotion along various physical structures. The practical importance of these studies lies in a detailed analysis of the experimental conditions and provides an objective description of cell locomotion which may be useful in the definition of granulocyte dysfunctions. Furthermore, the advantage of the technique employed is that it is not restricted to locomotion studies on granulocytes and is likely to be useful for similar investigations on other cell types, cell clusters or even whole animals.

II. Materials and methods

1. Polymorphonuclear leukocytes (Granulocytes)

Granulocytes were separated from heparinized venous blood of healthy human blood donors on a Ficoll-Hypaque density gradient as previously described (Zigmond 1978). After osmotic lysis of the remaining red blood cells the granulocytes were washed three times, and resuspended in Hanks' solution at a concentration of $4 \cdot 10^6$ cells/ml.

2. Chemokinetic factor

The chemokinetic substance Zymosan was derived from Yeast extract as previously described (Zigmond 1977).

3. Locomotion assay

The chambers were set up in analogy to the Zigmond chamber (Zigmond 1977). 50 µl of the cell suspension were pipetted on the substrate with specially prepared surface structures. The cells attached to the surface during an incubation time of 15 min at 37 °C in a humid incubator gassed with 5% CO_2 in air. The substrate with adherent cells was rinsed twice with Hank's buffer and was then loaded with a drop of Zymosan activated Hanks' buffer. The preparation was covered with a coverslip. The thickness of the aqueous layer was between 10 and 30 µm. Excess solution was removed by gentle suction. The coverslip was sealed to the substrate with paraffin wax to avoid loss of medium by evaporation. The loaded assay chamber was placed on a heated (37 °C) objective stage of a Zeiss photomicroscope. The preparation of the chamber resulted in an appropriate area cell density. At very low cell density a huge number of photographs are necessary and at very high cell density the cells are dense packed and no contact guidance can be observed.

4. Surface structures

The locomotion assay was built up with four different ground plates: *(i)* a glass plate from the Neubauer counting chamber, *(ii)* an optical grid, *(iii)* a machine drilled aluminium surface and *(iv)* a stretched polyethylene foil.

The granulocytes were studied in a sector of the Neubauer counting chamber where the diamond-cut grooves had a distance of 50 µm, a width of ≈ 2 µm, and a depth of 1 to 2 µm.

The ground plate of the optical grid was glass. The parallel lines of the optical grind consisted of chromium, which was deposited as a thin layer onto the glass. The periodic distance of the parallel lines was 5 µm. The boundary of the lines was very sharp.

The grooves in the aluminium surface were made with a drilling machine. The distance between the parallel lines was 11 ± 3 µm. The depth of the grooves was about 3 µm. The boundary of the grooves was not well defined since the grooves were fringed. The migrating cells were observed in reflected light (Nomarski differential interference contrast).

The stretched polyethylene foil was prepared in the following way: A 100 µm thick foil was stretched for 20 min in such a way that the original length, l, increased to 130% ($1.3 \cdot l$). After this procedure the foil was birefringent and the molecules were more or less in a parallel orientation. The apolar order parameter of all the macromolecules was about 0.3 (see Heise et al. 1977). The contact angle between a drop of water and the stretched polyethylene foil was angular dependent, indicating that the surface tension of the stretched material is anisotropic. The foil was also investigated with the scanning electron microscope. The surface became undulated after the stretching procedure, as shown in Fig. 1. The undulations were produced by an arrangement of short lines. The length of the lines was between 3 and 10 µm. The periodicity of the parallel lines was 0.5 to 1 µm.

The surface tension of the scratched aluminium surface, of the stretched polyethylene foil and of the

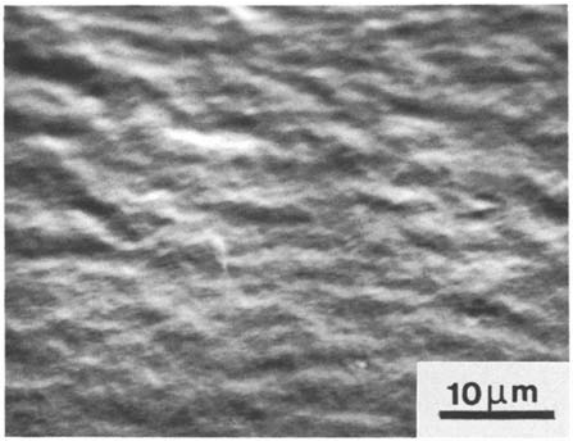

Fig. 1. Picture of a stretched polyethylene foil as seen in a scanning electron microscope. An arrangement of short lines becomes visible by shadowing the foil with gold-palladium

optical grid was angular dependent: a drop of water deposited on these surfaces, showed a shape which deviated from a circle. The contact angle measured between the water and the substrate was a function of the orientation with respect to the lines. The contact angle measured in the direction of the lines is larger than that measured perpendicular to the lines of the substrates.

5. *Temperature in the locomotion chamber*

The temperature of the assay had to be measured at the place where the cells were moving in order to avoid systematic errors since Nahas et al. (1971) showed that the track velocity of granulocytes depends very strongly on the temperature of the surrounding medium. The absolute temperature as well as the temperature distribution of the locomotion chamber were measured using a temperature sensitive liquid crystal mixture (Riedel de Häen AG). We used the following method: The assay was loaded with a temperature sensitive liquid crystal instead of the aqueous solution. The colour as well as the spatial distribution of the colour of the liquid crystal yielded information about the temperature and the temperature distribution in the locomotion assay. The relation between the reflection colour of the liquid crystal and the temperature was calibrated in a separate experiment. We used five different liquid crystal mixtures. The compositions are given in Table 1. With this technique we were able to adjust the locomotion assay to 37 °C and to measure the temperature distribution in the chamber to less than 1 °C.

6. *Microscopical observations and collection of data*

The motion of cells could be analyzed using time-lapse micrographs. The microscope was equipped with Nomarski optics and a picture was taken every 10 s.

Table 1. Temperature sensitive liquid crystal mixtures. The composition of the mixtures is given in weight parts. Each mixture changes its reflection colour from red to blue

Mixture number	1	2	3	4	5
Temperature range/°C	30–32	31–33	32–34	34–36	36–38
Cholesteryl-oleate	300	300	300	300	300
Cholesteryl-benzoate	50	50	50	50	50
Cholesteryl-chloride	40	40	40	40	40
Cholesteryl-pelargonate	80	100	120	150	165

The evaluation of the pictures was done in the following way:

(i) A picture was projected onto the graphic tablet of an Apple II computer. *(ii)* The graphic tablet was oriented in such a way that the x-axis was parallel to the lines on the different surfaces. *(iii)* The contour lines of the cell were determined by eye and then fed to the computer. *(iv)* The computer determined the central point and orientation of the cell from its contour line.

The static information which we obtained from a single picture could be translated into dynamic action using consecutive pictures of moving cells.

7. *Physical analysis of cell movement*

a) Area density distribution function

The area density distribution of the cells was determined in the case where the size of the cell was small compared to the grid distance (Neubauer counting chamber). The area density distribution was evaluated from photographs as follows: *(i)* The distance, L, between two lines of the periodic structure ($L = 50\,\mu m$) was separated into five equal segments with length Δy ($\Delta y = 10\,\mu m$). *(ii)* The number of cells in every segment was determined. *(iii)* The area density distribution, $N(y)$, was defined as the number of cells in every segment divided by the area of the segment.

It was not necessary to consider the distribution function of the whole viewing field since the assay was periodic. This means that every physical state, e.g. the average cell density at the position y, $N(y)$, is the same if the coordinates are changed in the y direction by the periodic length L.

$$N(y) = N(y \pm L). \tag{1}$$

This symmetry argument allowed us to project the parallel lines upon each other.

It was not necessary to consider the whole distribution between two lines since the y-axis could be exchanged for the $-y$ direction. It was enough to consider the cell density distribution of half a segment.

The measured cell density distribution, $N(y)$, is considered as the output signal of the cell. It can be expressed in a Fourier series:

$$N(y) = \sum_{k=0} [A_k \cos(2\pi k\, y/L) + B_k \sin(2\pi k\, y/L)]. \tag{2}$$

Because of the symmetry of the assay all B_k become zero. The Fourier series after the symmetry operation then reads

$$N(y) = A_0 + A_1 \cos(2\pi y/L) + A_2 \cos(4\pi y/L) + \ldots . \tag{3}$$

The Fourier coefficients $A_0, A_1, A_2, \ldots$ can be determined by fitting the experimental data to Eq. (3).

The response of the cells to an undulate surface can also be described by a density order parameter, $\langle P_{1d} \rangle$, if the first two Fourier coefficients are dominant:

$$\langle P_{1d} \rangle = A_1/A_0 . \tag{4}$$

Using the density order parameter, the cell density distribution may be rewritten as:

$$N(y) = A_0(1 + \langle P_{1d} \rangle \cos(2\pi y/L) + \ldots). \tag{5}$$

If the cells do not recognize the undulations of the surface, then A_1 as well as the density order parameter, $\langle P_{1d} \rangle$, would be zero. If, in contrast, the cells are attracted by the lines and concentrated on them, then A_0 would be equal to A_1 and the density order parameter, $\langle P_{1d} \rangle$, would be one. If the cells were repulsed from the lines and concentrated in the space between two lines, then A_1 would equal minus A_0 and the density order parameter would become minus one.

b) Angular distribution functions

i) The angular distribution function was determined in the case where the size of the cell was small as compared to the periodic length of the parallel lines in the following way: *(i)* Only cells which were in contact with one line were studied. *(ii)* The angle, α, between the long axis of the elongated cell and the line was measured. *(iii)* The number of cells having values of α in each of twelve 30° segments (−15° to 15°, ..., 315° to 345°) was used. *(iv)* The angular distribution function, $N(\alpha)$, was defined as the number of cells in every segment divided by the total number of counted cells.

It was not necessary to consider the distribution function of the whole viewing field since the assay was symmetric. The elements of symmetry of the assay could be determined in the following way: Supposing that an observer was looking at one side of a line and another observer at the opposite side then both would observe the same physical state. This means that the angular distribution function, $N(\alpha)$, measured by the first observer is indistinguishable from the angular distribution function, $N(\alpha \pm 180°)$, measured by the second observer.

$$N(\alpha) = N(\alpha \pm 180°). \tag{6}$$

This symmetry argument allowed us to project the angular distribution function for $\alpha > |\pm 90°|$ on to the angular distribution function for $\alpha \leqq |\pm 90°|$. The total number of cells in the considered segment increases by this procedure and therefore the statistical error decreases.

There was a further symmetry of the system: Supposing this time that one observer was looking in the $+\alpha$ direction and another observer in the $-\alpha$ direction, then both would observe the same physical state. This means again that

$$N(+\alpha) = N(-\alpha). \tag{7}$$

Again this symmetry argument allowed us to project the angular distribution function for $\alpha < 0°$ onto the angular distribution function for $\alpha \geqq 0°$.

The information which could therefore be obtained from the whole angular distribution function could also be obtained from the angular distribution for α between 0° and 90°.

ii) The angular distribution function was determined in the case where the size of the cell was large compared to the periodic length of the parallel lines in the following way: The direction of the cell locomotion, Θ_m, was determined by taking successive pictures.

$$\Theta_m = \arcsin(\Delta y_m(\Delta x_m^2 + \Delta y_m^2)^{-0.5}) \tag{8}$$

with

$$\Delta x_m = x(t_m) - x(t_{m-1}), \tag{9}$$

$$\Delta y_m = y(t_m) - y(t_{m-1}), \tag{10}$$

where $x(t_m)$, $y(t_m)$ and $x(t_{m-1})$, $y(t_{m-1})$, are the coordinates at times t_m and t_{m-1} respectively. The angular distribution function, $N(\Theta_m)$, was derived for different times t_m and different cells.

It was again only necessary to measure the angular distribution function between 0° and 90° because of the symmetry of the assay used.

iii) A time-dependent angular distribution function was determined in order to investigate short range order effects as the directional memory time of moving cells (Gruler and Bültmann 1984a). With measurement of this function, the following question could be answered: How fast does a cell lose the direction of migration when it has migrated for time t_m in a certain direction? A large amount of experimental data is necessary to construct time-dependent angular distribution functions for several directions. In this experiment, as there was not enough material to construct several of these functions, we neglected the anisotropy of the underlying surface.

The time-dependent angular distribution function was constructed for cells on the optical grid, the scratched aluminium surface and the polyethylene foil in the following way: *(i)* The direction of motion, Θ_m, was determined for the time t_m and the direction of motion, Θ_{m+k}, for the time t_{m+k}. *(ii)* The change in the direction of motion, $\Delta\Theta$ $(= \Theta_m - \Theta_{m+k})$, was determined for a fixed time interval t_k $(= \Delta t \cdot k)$. *(iii)* The angular distribution function was constructed for a fixed time interval (k = const). *(iv)* This procedure was repeated for different k-values ($k = 1, 2, 3, \ldots$).

Thus, for $k = 0$, the angular distribution function was a very sharp peak. It was one for $\Delta\Theta = 0°$ and zero

for all the other angles ($\Delta\Theta \neq 0°$). For $k = 1, 2, 3, \ldots$ the angular distribution functions deviate from the sharp peak. One would expect that the angular distribution function would broaden with increasing time and also it would show structural features for certain angles.

This change in the angular distribution function can be quantified by the polar order parameter, $\langle P_1 \rangle$ (Gruler and Bültmann 1984 a):

$$\langle P_1 \rangle = \langle \cos \Delta\Theta \rangle \ . \tag{11}$$

The polar order parameter normally quantifies the strength of the galvanotactic or chemotactic cellular response to a polar field, i.e. an electric field or a field created by the concentration gradient of chemotactic molecules (Gruler and Bültmann 1984 a, 1984 b, Bültmann and Gruler 1983). Here we used this parameter to determine the directional memory of the migrating cells. For $k = 0$, the average of $\cos \Delta\Theta$ was one ($\langle P_1 \rangle = 1$ for $t = 0$). For $k \neq 0$, the angular distribution function was a structured function and the polar order parameter was therefore smaller than one. How the polar order parameter decays in time would describe the directional memory of the cell (Gruler and Bültmann 1984 a).

iv) In this section the short range order effects were investigated. The question was as to how the anisotropic substrate alters the cellular response? To answer this question, we used the following procedure: *(i)* At time t_m, the direction of migration was determined. *(ii)* The whole plane was separated into equal angular segments (see II.7.a). *(iii)* The cells for every segment were determined and regarded as a group. *(iv)* The direction of locomotion, Θ_{m+1} for t_{k+1} and the change of direction $\Delta\Theta$ were determined. *(v)* The cells of every group were separated into two subgroups according to whether they changed their direction clock-wise or counter clock-wise. *(vi)* The average angle of the change of migration was determined for every subgroup.

c) Virtual force and torque

The cells were attracted by the lines in the anisotropic environment. The virtual force and torque can be determined by the following steps: First, the distribution which characterizes the cellular response is interpreted by its generating function (Haken 1977). Second, the generating function is considered as a normalized potential. Third, the force and torque are calculated from the potential (Haken 1977).

i) Generating functions. Step 1: Cellular responses resulting in attraction and orientation by the lines are described by the cell area density distribution function, $N(y)$, and the angular distribution function $N(\Theta_m)$. These distribution functions are always positive, therefore they can be expressed by new functions, $V(y)$ and $V(\Theta_m)$, without losing information (Haken 1977).

$$N(y) \ = \mathrm{e}^{V(y)}, \tag{12}$$

$$N(\Theta_m) = \mathrm{e}^{V(\Theta_m)}. \tag{13}$$

Step 2: The generating function has the meaning of a normalized energy. This can be illustrated by the following example: The density of molecules, n, in the atmosphere is a function of altitude, h. The distribution of the density of the molecules in the gravitational field is described by Boltzmann statistics as:

$$n(h) = n(0)\, \mathrm{e}^{-\mathrm{mgh/(kT)}}. \tag{14}$$

The potential energy is mgh, where m, g, and kT are the mass of the molecule, the acceleration due to gravity, and the thermal energy. $n(0)$ is the density of the molecule at the ground. The generating function is then defined as:

$$V(h) = \ln n(0) - \mathrm{mgh/(kT)}. \tag{15}$$

Step 3: The thermodynamic force can be calculated from the known potential, $V(h)$, as

$$F = -\,\mathrm{d}V(h)/\mathrm{d}h = \mathrm{mg/(kT)}. \tag{16}$$

The virtual force, $F(y)$, and the virtual torque, $M(\Theta_m)$, can be obtained in analogy with Boltzmann statistics:

$$F(y) \ = -\,\mathrm{d}V(y)/\mathrm{d}y, \tag{17}$$

$$M(\Theta_m) = -\,\mathrm{d}V(\Theta_m)/\mathrm{d}\Theta_m. \tag{18}$$

The virtual force has the dimensions of inverse length, while the virtual torque has no dimensions.

d) Velocity of the cells

Exposure of the cells to chemokinetic stimuli results in active movement, accompanied by a change of starting position, with a certain speed.

The mean velocity, $\langle v_x \rangle$ and $\langle v_y \rangle$ are zero since the mean displacements, $\langle \Delta x \rangle$ and $\langle \Delta y \rangle$, are zero because of the symmetry of the assay used [see Eqs. (6)–(7)].

Quantities which are not equal to zero are the mean square velocities defined as

$$\langle v_x^2 \rangle = N^{-1} \sum_{m=1}^{N} (\Delta x_m/\Delta t)^2, \tag{19}$$

$$\langle v_y^2 \rangle = N^{-1} \sum_{m=1}^{N} (\Delta y_m/\Delta t)^2. \tag{20}$$

The mean square track velocity, $\langle v_c^2 \rangle$ was calculated as

$$\langle v_c^2 \rangle = N^{-1} \sum_{m=1}^{N} (\Delta x_m^2 + \Delta y_m^2)/\Delta t^2. \tag{21}$$

In this article the square root of the mean square track velocity, $(\langle v_c^2 \rangle)^{0.5}$, will be referred as the "track velocity", v_c, of the cells.

The mean square velocity for any direction, ϕ, can also be obtained from successively taken coordinates of the cells (rotation of the coordinate system by the angle ϕ):

$$\langle v(\phi)^2\rangle = N^{-1}\sum_{m=1}^{N}(\Delta x^2\cos^2\phi + \Delta y^2\sin^2\phi)/t^2. \quad (22)$$

In our experiment, the data were obtained and evaluated for $\phi = 0°$, 30°, 60°, and 90°.

e) Mean square displacement of cells

Active cell movement was characterized by the mean square velocities. Here we describe another possible way of quantifying this process by considering mean square displacements.

The mean displacements, $\langle\Delta x\rangle$ and $\langle\Delta y\rangle$, are zero as already mentioned. This means that all cells move but that the centre obtained from cells does not change in space. To quantify the random walk process of the migrating cells one has to take the mean square displacements which are defined as:

$$\langle x(t)^2\rangle = N^{-1}\sum_{i=1}^{N}(x_i(t_m) - x_i(t_0))^2, \quad (23)$$

$$\langle y(t)^2\rangle = N^{-1}\sum_{i=1}^{N}(y_i(t_m) - y_i(t_0))^2. \quad (24)$$

A short theoretical consideration of the mean square displacement: The random movement of particles, atoms, molecules or even animals is described in a general way by the diffusion equation, developed by Einstein (1905).

$$\langle x(t)^2\rangle = 2D_x t, \quad (25)$$

$$\langle y(t)^2\rangle = 2D_y t. \quad (26)$$

The mean square displacement of moving particles $\langle x^2\rangle$ and $\langle y^2\rangle$ are proportional to the time $t\,(=t_m - t_0)$. D_x and D_y are diffusion coefficients. Fürth (1920) extended this equation by introducing the characteristic time, τ, which is involved in locomotion and got the following equation:

$$\langle x(t)^2\rangle = 2D_x(t - \tau(1 - e^{-t/\tau})), \quad (27)$$

$$\langle y(t)^2\rangle = 2D_y(t - \tau(1 - e^{-t/\tau})). \quad (28)$$

These equations can be simplified for $t > \tau$ as follows:

$$\langle x(t)^2\rangle = 2D_x(t - \tau), \quad (29)$$

$$\langle y(t)^2\rangle = 2D_y(t - \tau). \quad (30)$$

If one plots the mean square displacement as a function of time t then one would expect a straight line for $t > \tau$. Its slope would be given by the diffusion coefficient D_x (or D_y) and its intercept with the t-axis would determine the characteristic time τ. The values of the diffusion coefficients and the characteristic time were actually obtained by fitting Fürth's diffusion equation to the experimental data. The fitting parameters were the diffusion coefficient and the characteristic time τ.

For any direction, ϕ, the diffusion coefficient can be obtained by rotating the coordinate system. Fitting Fürth's equation to the experimental data yields the diffusion coefficient $D(\phi)$ and the characteristic time τ.

The diffusion coefficients, $D(\phi)$, determined for the different angles can be expressed by a Fourier series:

$$D(\phi) = \sum_{k=0}(D_{k,g}\cos k\phi + D_{k,u}\sin k\phi). \quad (31)$$

Because of the symmetry of the assay one has the following relations

$$D(+\phi) = D(-\phi), \quad (32)$$

$$D(\phi) \quad = D(\phi \pm 180°). \quad (33)$$

$D_{k,u}$ is zero for every k and $D_{k,g}$ is zero for odd k. The Fourier series after the symmetry operation would be:

$$D(\phi) = D_{0,g} + D_{2,g}\cos 2\phi + D_{4,g}\cos 4\phi + \ldots. \quad (34)$$

The Fourier coefficients $D_{0,g}, D_{2,g}, D_{4,g}, \ldots$ were determined by fitting the experimental data to Eq. (34).

The response of the cells to the undulate surface can also be expressed by an apolar order parameter, $\langle P_2\rangle$, if the first two Fourier coefficients, $D_{0,g}$, $D_{2,g}$, are dominant:

$$\langle P_2\rangle = D_{2,g}/D_{0,g}. \quad (35)$$

The response of moving cells to an undulate surface can now be described by

$$D(\phi) = D_{0,g}(1 + \langle P_2\rangle\cos 2\phi + \ldots), \quad (36)$$

This equation describes an ellipse. The diffusion coefficients in the x- and y-direction are

$$D_x = D_{0,g} + D_{2,g}, \quad (37)$$

$$D_y = D_{0,g} - D_{2,g}. \quad (38)$$

If the cells did not recognize the undulations of the surface, then $D_{2,g}$ as well as the apolar order parameter, $\langle P_2\rangle$, would be zero and D_x would equal D_y. In this case the random walk is isotropic and $D(\phi)$ is described by a circle. If D_y is zero and D_x unequal to zero, then all the cells migrate parallel to the lines of the substrate and the apolar order parameter, $\langle P_2\rangle$, would become one. If D_x is zero and D_y unequal to zero then all the cells migrate perpendicular to them and the apolar order parameter would become minus one. In migration patterns between these two extremes we can describe the anisotropy of the random walk process on a substrate with an apolar symmetry by the deviation of the diffusion coefficient $D(\phi)$ from the circle with radius $D_{0,g}$.

f) Gain of information

One has the following general phenomenon: In isotropic surroundings the locomotion of cells is disordered and in anisotropic surroundings it is expected to be somehow ordered. This means that information would be involved in cellular response. One could ask how much information would be necessary for a disordered movement to be transformed into an "ordered" one. This question can be answered by evaluating the distribution functions since they quantify the cellular respone. The amount of information which the cells collect from their environment must be hidden in these distribution functions.

The information which is necessary to make out of the distribution function p' a new distribution p, is the gain of information, $K(p';p)$. Rényi (1970) derived the following equation (see also Haken 1977; Schlögel 1971):

$$K(p';p) = \sum_{i=1}^{N} p_1 \log_2 (p_i/p_i'). \tag{39}$$

The gain of information is written in such a way that the result is given in bits. A value of one bit means: To *one question, one answer* with yes or no.

Gain of information for cells in contact with either a single line or many parallel lines when the size of the cell is large in relation to the periodic length of the assay: If the cells ignore the line(s) or if there are no lines at all, then the angular distribution function would be a constant ($N_{iso} = p' = \text{const}$). However, if the cells recognize the line(s) and collect some information, the angular distribution function would not be a constant ($N_{angle} = p \neq \text{const}$). By applying Eq. (39) one obtains the information which the cells could collect from the line(s) in order to orient themselves along it or them.

III. Results

1. Small cell size in relation to grid distance

a) Cell density distribution. Cell locomotion in a Neubauer counting chamber where the cell size is small compared to the grid distance, was investigated. The distribution function of the area density of the cells was determined immediately after exposure of the cells to the surface and after 1 min, 10 min, and 20 min. After 1 min, the cells were more or less homogeneously distributed all over the surface as shown in Fig. 2a. There was no evidence that the lines played a significant role in the adhesion process during sedimentation. The distribution function of the cell density was constant, independent of the position on the surface. Obviously the cells had not reached the steady state condition. After 10 min, the cell density at the lines was larger when compared to that of the flat surface of the glass (Fig. 2b). Evaluation of the photographs yielded the distribution function of the cell density (Fig. 3a). The cells photographed after 20 min yielded the same behaviour as those after 10 min which indicates that the cells are already in steady state after 10 min.

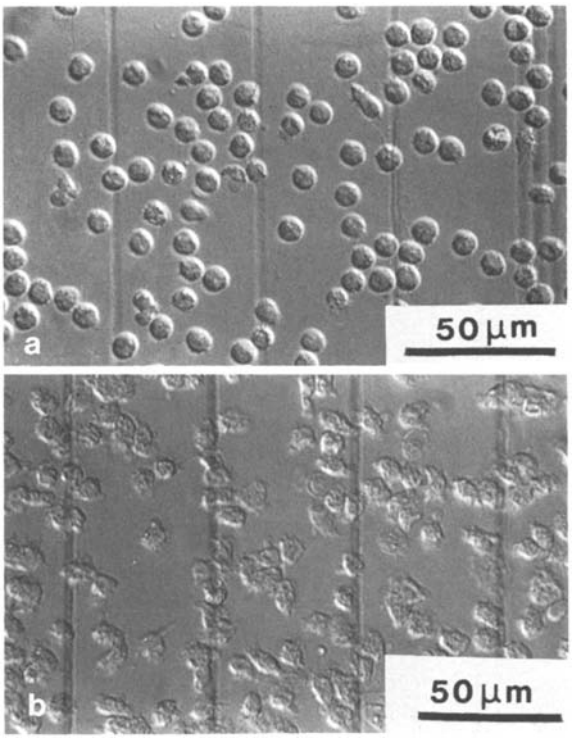

Fig. 2a and b. Microscope pictures (Nomarski) of moving cells on a ground plate of a Neubauer counting chamber. **a** and **b** shows the cells distribution 1 and 10 min after application the cells to the surface

These investigations have allowed us to conclude that the distribution function of the cell density is anisotropic. The amount of information which the cells oriented from the lines can be calculated according to Eq. (39). The result was 0.04 bits.

b) Angular distribution along a line. We found that the orientation of the cells which were in contact with the lines was anisotropic. The angular distribution function of these cells was 0.34, 0.19, 0.19, and 0.27 for the angular segments (−15° to 15°), (15° to 45°), (45° to 75°), and (75° to 105°), respectively. The angular distribution function was anisotropic and it is likely that the cells had collected information from the lines on the surface. The amount of information collected was 0.03 bits.

2. Large cell size in relation to the grid length

Cell movement was investigated on an optical grid, a scratched aluminium surface, and a stretched poly-

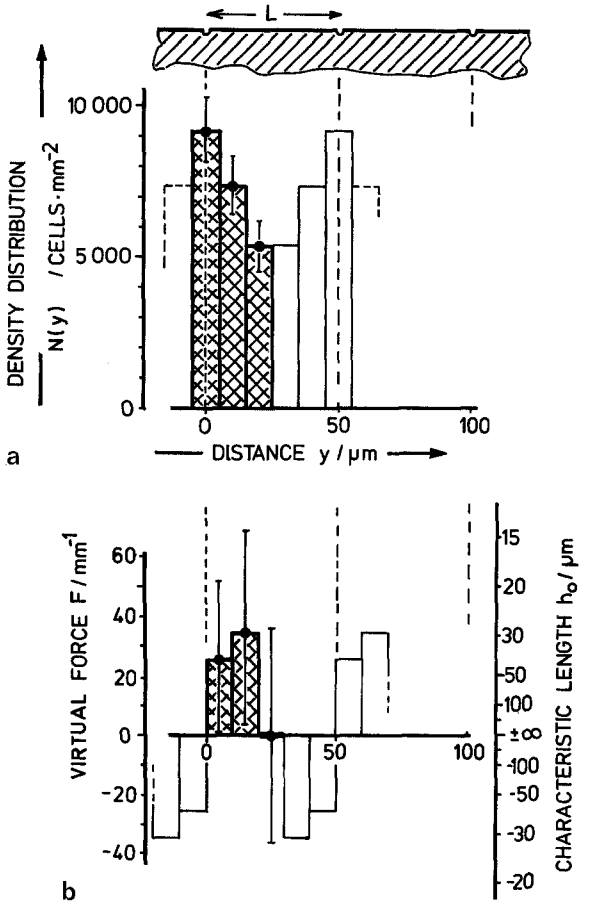

Fig. 3. a The area density distribution of cells 10 min after application on a Neubauer counting chamber. The cell density is highest at segments containing lines and lowest in segments containing only flat surface areas. **b** The virtual force is demonstrated as a function of cell localisation on the surface of the Neubauer counting chamber. The direction of this virtual force always acts towards the lines

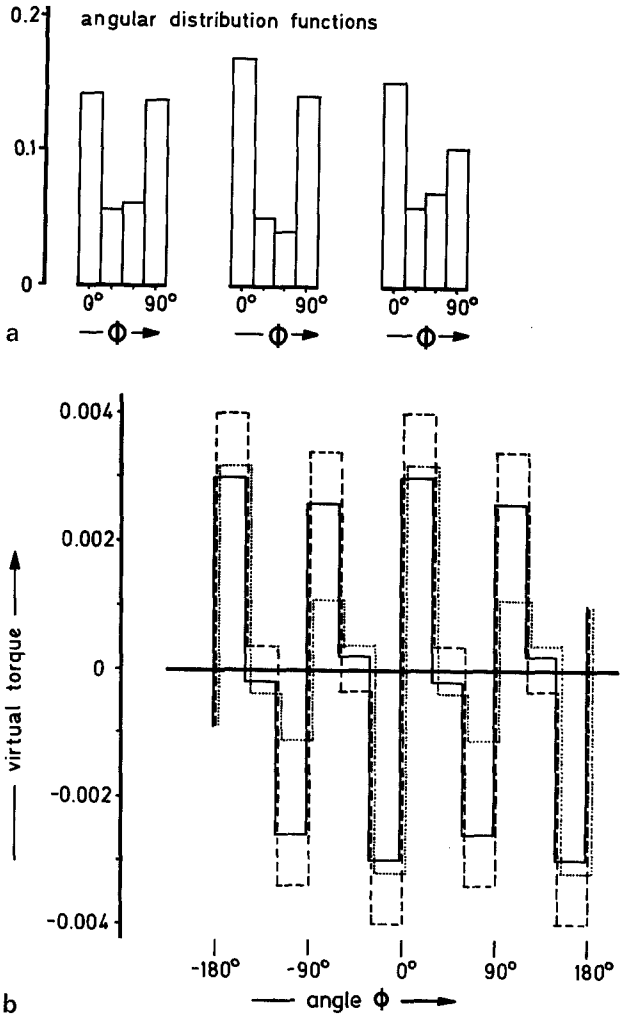

Fig. 4. a The angular distribution function is shown for cells migrating on three different surfaces (from *left to right:* stretched polyethylene foil, scratched aluminium plate, optical grid). The ordinate is the number of cells in an angle sector divided by the total number of counted cells. **b** The virtual torque derived from the angular distribution functions are shown for different surfaces: optical grid (*full line*), scratched aluminium plate (*dotted line*) and stretched polyethylene foil (*dashed line*)

ethylene foil. Appropriate distribution functions and the information which the cells collected were determined.

a) Angular distribution function. The anisotropic locomotion of cells on an apolar substrate was quantified by determination of the angular distribution functions (Fig. 4). These functions were anisotropic and, therefore, the cells obtained information from the modulated surfaces. The amount of information was 0.12, 0.25, and 0.12 for the optical grid, the scratched aluminium surface, and the stretched polyethylene plate, respectively.

b) Cell velocity. The chemokinetic response of the cells was investigated by measuring their track velocity. The result was a broad distribution (Fig. 5). A similar broad distribution was measured in the case of a flat glass surface (Haston et al. 1982; Gruler and Bültmann 1984a). The average track velocity $(\langle v_c^2 \rangle)^{0.5}$, with its standard deviation was 0.68 ± 0.11, 0.81 ± 0.12, and 0.71 ± 0.11 µm/s for the optical grid, the scratched aluminiun surface, and the stretched polyethylene foil, respectively. These average values were obtained when the anisotropy of the substrate was neglected.

The velocity in different directions was determined according to Eq. (22) (Table 2). We observed that the mean velocity was a function of the direction: It was highest parallel to the lines and lowest perpendicular to them. Similar results were found for all substrates investigated. The square root of the mean square velocity as a function of $\cos 2\phi\,(=2\cos^2\phi - 1)$ can be very well approximated by a straight line. The correlation coeffficient is better than 0.95.

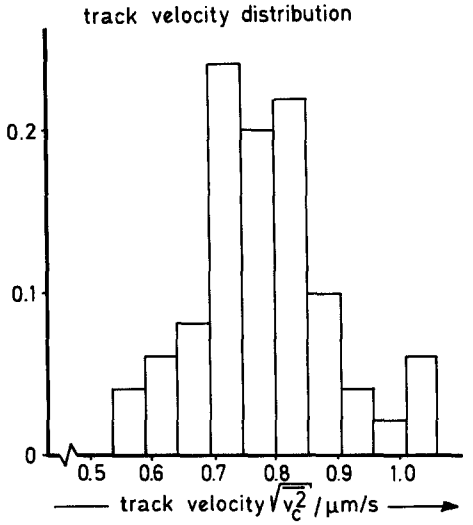

Fig. 5. The "track velocity" distribution of cells moving on an aluminium surface

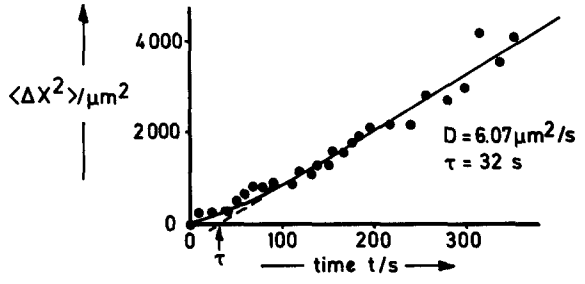

Fig. 6. The mean square displacement of cells on a scratched aluminium plate as a function of time ($\phi = 90°$). The experimental data approximately described a straight line for the time-interval measured. The values of the diffusion coefficient D and the characteristic time τ may be determined from the slope and the intersection with the t-axis of the straight line (*dashed line*). The actual values of D and τ where obtained by fitting Eq. (27) to the experimental points (*full line*)

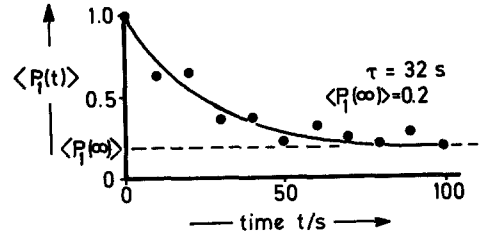

Fig. 7. The time dependence of the polar order parameter for cells migrating on an optical grid is shown. The result is an exponential curve, defined by a constant τ', the characteristic or "memory" time of the migrating cells

c) Random walk and internal clock. Non-directed cell locomotion could be quantified by the mean square displacements as a function of time. By using Eqs. (27) and (28), the mean square displacement could be determined from the coordinates of the cells (Fig. 6). The points were obtained by evaluation of the photographs. The thick line is a fit of Eq. (27) or (28) to the points. The fitting parameters are the diffusion coefficient, D, and the characteristic time, τ. The values of the diffusion coefficient are shown in Table 3. The main results were: *(i)* The diffusion coefficient was a function of the substrate used. *(ii)* The diffusion coefficient for one substrate could not be described by one value since D depended on the angle of observation. *(iii)* The diffusion coefficients were maximum parallel and minimum perpendicular to the lines. They could be used to characterize the anisotropic random walk.

Table 2. Angular dependence of the square root of the mean square velocity $\sqrt{v(\phi)^2} \pm \sigma$ μm s^{-1}

Angle ϕ	Optical grid	Scratched aluminium plate	Stretched polyethylene plate
0°	0.47 ± 0.07	0.52 ± 0.08	0.48 ± 0.08
30°	0.46 ± 0.07	0.49 ± 0.08	0.46 ± 0.07
60°	0.41 ± 0.06	0.44 ± 0.07	0.45 ± 0.07
90°	0.35 ± 0.05	0.44 ± 0.07	0.42 ± 0.06
Correlation coefficient to a straight line $\sqrt{v(\phi)^2} = A_v + B_v \cdot \cos 2\phi$			
	0.96	0.97	0.95

Table 3. Angular dependence of the diffusion coefficients

Angle ϕ	Optical grid	Scratched aluminium plate	Stretched polyethylene foil
0°	6.40	6.07	5.30
30°	6.05	5.71	4.45
60°	4.71	3.71	3.35
90°	3.46	2.70	3.04

d) Directional memory. The directional memory of a cell could be obtained if the time-dependent angular distribution function were determined. The temporal change of these distribution functions was quantified by temporal changes of the polar order parameter, $\langle P_1(t) \rangle$ (Fig. 7). The polar order parameter decreased with increasing time. This decay could be described by an exponential law with a characteristic time constant of 32 s (=memory time). Cells exposed to an undulate surface also have a long term memory, as indicated by the finite value of the polar order parameter for long time intervals, $\langle P_1(\infty) \rangle$. This long term memory time induces another persistence mode in the random walk. The theoretical prediction for $t > \tau$ is

$$\langle x(t)^2 \rangle = f \langle v_x^2 \rangle \langle P_1(\infty) \rangle t^2, \tag{40}$$

where f is a factor. The contribution of the long term memory to the random walk must be very small since

Table 4. Average angular change in direction of migration as a function of cell orientation

Previous direction of migration	Average angular change in direction		
	Clock-wise	Counter clock-wise	
$[\Theta_m]$	$[\alpha^-]$	$[\alpha^+]$	$[\Theta_m - \alpha^+]$
15°–25°	109°	29°	− 9°
25°–35°	126°	34°	− 4°
35°–45°	163°	52°	−12°
45°–55°	129°	60°	−10°
55°–65°	75°	63°	− 3°

the experimental results as shown in Fig. 6 are very well described by the Langevin equation (Eq. 27 or 28). It is not necessary to enlarge the Langevin equation by Eq. (40).

e) Anisotropy of the change in direction. The average change in direction was determined for cells which turn clock-wise, α^-, as well as for cells which turn counter clock-wise, α^+. This angular change was measured as a function of the previous direction of locomotion, Θ_m (Table 4). We observed that the cells tended to change their direction in such a way that afterwards they migrated parallel to the lines. This effect could best be seen if the angle of the previous direction of locomotion, Θ_m, was subtracted from the average angular change of locomotion, α. $(\Theta_m - \alpha^+)$ is about zero. There are deviations from this law at angles $\Theta_m = 0°$ and $\Theta_m = 90°$.

IV. Discussion

1. Recognition

Numerous workers (Harrison 1912; Weiss 1959, 1961; Weiss and Garber 1952; Dunn and Ebendal 1978; Dunn and Heath 1976; Ebendal 1977; Lackie and Wilkinson 1984; Haston et al. 1982) have demonstrated that granulocytes and other cell types (e.g. fibroblasts) can recognize different stuctural details of their surroundings. In the present investigation we have quantified this ability in granulocytes: First, the cell density was measure and found to be larger at the lines than at the flat areas of the substrate. Second, the orientation along a single line was measured and the angular distribution function was shown to be anisotropic. Third, the diffusion coefficients and track velocities were found to be anisotropic too. If the cells were not aware of structural details, all the above determined functions and parameters would be constant. However, the exact nature of this "awareness" is still unknown, though certain characteristics of this mechanism are evident:

(i) It could be suggested that the cell detects structural details via a membrane bound receptor. However, this is very unlikely since a single receptor is much too small compared to the extension of an undulate surface. This problem could be solved if the receptors interact with each other. In this case, the effective size of the receptors could be as large as the size of the cells itself. This concept, based on an analogy to chemotaxis, seems very unlikely, because a highly specialised receptor can hardly account for cellular response to different surfaces, i.e., optical grid, scratched aluminium surface, etc.

(ii) The basis of the detection of structural details could be on the pattern of forces which are involved in galvanotaxis. Details of the galvanotactic response are not known. In any case, if the cell has to measure the electric as well as the dielectric properties of its surroundings, a mechanism for detecting the electric field in extracellular space is necessary. The existence of such an extracellular field has not been demonstrated for granulocytes, but Nuccitelli (1983) has noted the presence of such an extracellular field for many different large cells. For example, the formation of a leading front in a moving amoeba is connected to the formation of an extracellular field.

(iii) Another basis for detecting structural details could be a system like that which acts in the process of cell adhesion. It is well known that surface tension, which characterizes cell adhesion, is altered if a substrate is altered in shape: for example from a flat surface to a curved one. This model has been discussed by Weiss (1959, 1961).

(iv) The last possible basis could be elastic strain fields in the plasma membrane which are induced by bending the membrane. This would result in different chemical decomposition depending on the degree of bending of the membrane (Gruler 1975; Marcerou et al. 1984).

The leading front, as an essential element in the recognition process, can be distinguished morphologically from the rest of the plasma membrane, suggesting that molecular decomposition does take place. The origin of this decomposition is not clear. It could be an elastic strain field (point iv), or a change in the surface tension of the substrate (point iii), or an electric field (point ii). It is necessary to have more experimental data to differentiate between these different phenomenon.

In order to decide between these useful groups of hypotheses about the contact guidance behaviour we must turn to theoretical criteria and one such is the possible information content of the enviroment (point 2 in the discussion), and another one is the input-output analysis of the cellular response (point 3 in the discussion). All our results of these investigations are concentrated in point 5 of the discussion.

There we will show experimental evidence which support the observations of Weiss. There is a further guiding line through the discussion. We will mention from time to time the bridges connecting physics with biology.

2. *Gain of information*

In physical systems, such as gas molecules in a closed box, the final state is such that the entropy is maximum. The system is then expected to be the most probable state – the disordered one. More order can be induced by communicating with the molecules of the system. A simple process of communication is to just apply a force. The consequence would be that the density of the molecules would become space dependent, that the entropy of the system would decrease and that the system would therefore become more ordered.

In general we may say that any physical and biological system can deviate from maximum entropy if it had an interaction with its surroundings that resulted in transmission of information to the system. The interaction may take place as the result of immediately available information, or the information may originate from past situations where heredity and the memory of the system were important.

The amount of information which cells have collected from their environment was determined. Granulocytes moving in a concentration gradient of chemotactic molecules (10 nM/mm f-Met-Leu-Phe) collected a surprisingly low amount of information (≈ 2 bits) (Gruler and Bültmann 1984a; Gruler and Nuccitelli 1985). Granulocytes exposed to an undulate surface collected even a lower amount, 0.04 to 0.3 bits. The directional memory was very short. From the charactristic time of the moving state it seems that the measured gain of information is essentially that which is immediately available. This amount depends on the line density; the greater the number of lines recognized by the cell, the better the existing order. This fact in analogous to chemotaxis and galvanotaxis, where increasing the strength of the polar fields leads to better order in the corresponding systems.

The migrating cell is a molecular machine with very complex biochemistry. The principles that guide this molecular machine in the case of an anisotropic substrate may be very simple since the cellular response requires so little information. In point 5 we will argue that the basis for contact guidance is the anisotropic surface tension of the substrate.

3. *Input-output analysis*

We have demonstrated that moving cells have the ability to collect information from their environment. This ability can be discussed in more detail if a system analysis is performed: A cell can be considered as a "black box" which has an input channel for information from the surroundings and an output channel which represents the reaction of the cell to the input signal. In this model, it is not necessary to know the details of the "black box". However, it is necessary to quantify the input signal as well as the output signal. The following discussion splits into two parts: The cell size is either large or small in relation to the grid distance.

a) Cell size large in relation to the grid length

(i) Input signal: The signal which a cell obtained to direct its movement originated from the undulate substrate. The following hypotheses can be put forward: *(i)* Cells could determine their position in space. This seems very unlikely. *(ii)* The slope of a surface could be determined by a cell if it has devices which are sensitive to gravitation of forces. Heavy particles floating in the cytoplasm, which can influence the decision process of the cell, could be such a measuring device. However, the response time of such a system is large ($\gg 1$ min). It may not respond within the short characteristic time of locomotion (about half a minute). *(iii)* The curvature of a substrate could be determined by a single cell. The physical basis of such a system could be the surface tension, an electric field, or an elastic strain field leading to an altered molecular composition of the plasma membrane. Such a molecular process in the plasma membrane can occur very fast: i.e. an area of 1 μm^2 can be rearranged within 8 s taking a diffusion coefficient of $10^{10}\,cm^2/s$. This value is typical for membrane bound proteins (in a fluid membrane) (Peters 1981).

(ii) Output signal: The anisotropic random walk process is the output signal of the cell and can be quantitated by the angular dependent diffusion process.

The Fourier coefficients $D_{0,g}, D_{2,g}, D_{4,g}, \ldots$ were determined by fitting the experimental data to Eq. (35). For this purpose the measured diffusion coefficients $D(\phi)$ were plotted against $\cos 2\phi$ (Fig. 8). The data are approximately distributed along a straight line, indicating that only $D_{0,g}$ and $D_{2,g}$ are important for this process. The slope of the straight line determines $D_{2,g}$ and the diffusion coefficient $D(0)$ determines the values for $D_{0,g} + D_{2,g}$. The higher order terms $D_{4,g}, D_{6,g}, \ldots$ must be very small since they are determined from the deviation from straight line behaviour. This means that the anisotropic random walk process on a substrate with an apolar symmetry is analogous to the diffusion process of molecules in an apolar symmetric surrounding. A typical example is the diffusion of a molecule in a nematic liquid crystal (de Gennes 1974).

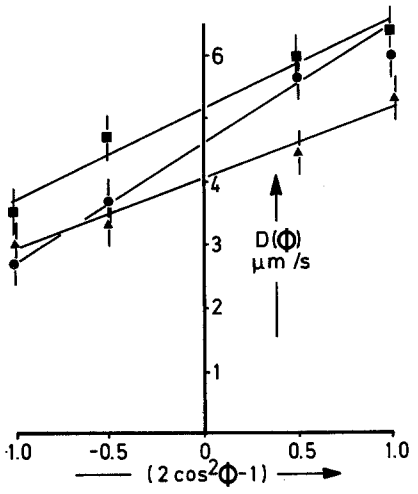

Fig. 8. Nematic type of contact guidance: The angular dependence of the diffusion coefficients for cells migrating on different substrates (optical grid ■, scratched aluminium plate ●, and streched polyethylene foil ▲). The straight line behaviour indicates that the cells do not recognize all the detail of their surrounding

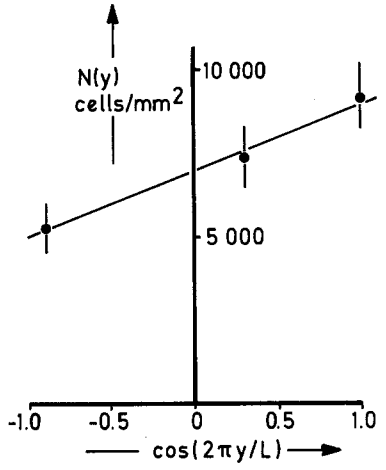

Fig. 9. Smectic type of contact guidance: The spatial dependence of the area density distribution function for cells migrating on the ground plate of a Neubauer counting chamber. The straight line behaviour indicates that the cell can only recognize a blurred surrounding

The anisotrpic random walk is characterized by the apolar order parameter, $\langle P_2 \rangle$. The exact value of $\langle P_2 \rangle$ can be determined either by following the cells in time or by measuring the orientation of the moving cells at fixed time. The evaluation of the dynamic measurements yields the anisotropic diffusion process. The apolar order parameter $\langle P_2 \rangle_{\text{diff}}$ calculated from the diffusion coefficients was 0.28, 0.40, and 0.25 for cells migrating on an optical grid, scratched aluminium plate and a stretched polyethylene plate, respectively. $\langle P_2 \rangle$ may also be determined through the angular distribution function $N(\phi)$ if the cells are elongated in the direction of their movement as

$$\langle P_2 \rangle_{\text{orient}} = 2 \langle \cos^2 \phi \rangle - 1 , \tag{41}$$

$\langle P_2 \rangle_{\text{orient}}$ was 0.09, 0.08, and 0.07 for cells oriented by the optical grid, the scratched aluminium plate, and the stretched polyethylene plate, respectively. $\langle P_2 \rangle_{\text{orient}}$ was much smaller than $\langle P_2 \rangle_{\text{diff}}$, indicating the orientation of the cells was not only induced by the directed locomotion. The main deviation between these static and dynamic measurements lay in the peak at $\pm 90°$ of the angular distribution function. By omitting this peak, $\langle P_2 \rangle_{\text{orient}}$ became 0.31, 0.36, and 0.27 for cells oriented by an optical grid, a scratched aluminium plate, and a stretched polyethylene plate, respectively. The similarities between dynamic and static measurements show that cells on an undulate surface are essentially oriented in the direction of their locomotion as on a flat substrate. However, the undulations affect and alter this cell orientation. This becomes most evident at $\pm 90°$.

In conclusion, we may say that the output signal of a moving cell on an undulate substrate is described by the apolar parameter, $\langle P_2 \rangle$, which can be determined either by the diffusion constants or by the angular distribution function if the values at $\pm 90°$ are neglected.

b) Small cell size in relation to grid length

In this case, the input signal (the grid length of the undulate surface) is large compared to the size of the moving cell. In the assay employed, the grooves on the glass plate were far apart and separated by a flat surface. A Fourier series of such an undulate surface has many terms. To avoid a description involving so many terms we used another approach to define the scratched glass surface: The grooves were compared to half of a cylinder with radius R. Consequently the curvature of a groove was R^{-1}. In the case of the Neubauer counting chamber, R was about 1 μm and, therefore, the curvature 1 μm^{-1}. The curvature of the flat part of the glass surface was zero. Thus it became possible to characterize the input signal by sharp spikes.

The cell used this input signal to guide its movement in such a way that the random walk activity depended on its position on the substrate. Thus the cell density became space dependent. The corresponding area density distribution, $N(y)$, would be considered as the output signal of the cell. It can be expressed in a Fourier series (Eq. (2)). The Fourier coefficients $A_0, A_1, A_2, \ldots$ were determined by fitting the experimental data to Eq. (3). For this purpose, the measured cell area density was plotted against $\cos(2\pi y/L)$ as shown in Fig. 9. The values distributed themselves along a straight line, indicating that only A_0 and A_1 were important for this process. Consequently, the

higher order terms are likely to be very small since they are determined from a deviation from straight line behaviour. The slope of this straight line determines A_1, and the intercept for $\cos(2\pi y/L) = 1$ determines A_0. The coefficients A_0 and A_1 for granulocytes moving on a ground plate of a Neubauer counting chamber were 7,400 cells/mm^2 and 1,500 cells/mm^2, respectively.

The response of the cells to an undulate surface can also be described by a density order parameter, $\langle P_{1d} \rangle$ (Eq. (4)). The density order parameter quantifies how strongly cells are attracted or repulsed by the lines. For example, the density order parameter for migrating granulocytes on the ground plate of a Neubauer counting chamber was +0.20.

As described above for the apolar order parameter, $\langle P_2 \rangle$, the density order parameter, $\langle P_{1d} \rangle$, may be determined either by measuring the position of the migrating cell in time or by measuring the position of many moving cells at a fixed time. In the first case, the time average of $\cos(2\pi y(t)/L)$ has to be calculated

$$\langle P_{1d} \rangle_{\text{time}} = \langle \cos(2\pi y(t)/L) \rangle_{\text{time}}, \tag{42}$$

and in the second case the average of many cells at a fixed time has to be calculated.

$$\langle P_{1d} \rangle_{\text{cells}} = \langle \cos(2\pi y/L) \rangle_{\text{cells}}. \tag{43}$$

We observed the same results employing both methods.

We observed that the cells were attracted by the grooves when the cell size was small compared to the grid length of the substrate. This phenomenon can be described by a virtual force. When the cell size was large compared to the grid length of the substrate, we observed that there was a tendency for the cells to be suspended either parallel or perpendicular to the lines. This phenomenon can be described by a virtual torque.

The virtual force of granulocytes moving on a ground plate of a Neubauer counting chamber is shown in Fig. 3 b. The virtual torque as a function of cell orientation is shown in Fig. 4 b, for cells moving on an optical grid, a scratched aluminium plate and a stretched polyethylene foil. This virtual torque acted on the cells and forced them to orient parallel to the lines. It was seen around 0° and ± 180° with nearly the same magnitude for the three different substrates. A further torque orients the cells perpendicular to the lines. This torque was pronounced for the aluminium plate and the stretched polyethylene foil and small for the optical grid. It is seen around ±90° and may be explained by the imperfection of the lines in the substrate. The lines of the optical grid had sharp corners but the lines in the aluminium were fringed and the stretched polyethylene foil had an undulate surface consisting of an arrangement of short lines.

We now want to show that a direct relationship exists between the above defined virtual force and torque and the earlier description of the cellular response by a Fourier series.

It is simple to demonstrate such a relationship since we found experimentally that the Fourier series consisted only of two dominant terms. In the case of small values of the order parameters ($\langle P_{1d} \rangle < 1$, $\langle P_2 \rangle < 1$), the generating functions are

$$V(y) = \ln A_0 + \langle P_{1d} \rangle \cos(2\pi y/L) + \ldots, \tag{44}$$

$$V(\Theta_m) = C_0 + \langle P_2 \rangle (2\cos^2 \Theta_m - 1) + \ldots, \tag{45}$$

where C_0 is a constant. Its value can be determined by integrating the angular distribution function $N(\Theta_m)$ and setting the result equal to one. Thus the generating functions, $V(y)$ and $V(\Theta_m)$, are described by the terms. The virtual force and torque are

$$F(y) = (2\pi y/L) \langle P_{1d} \rangle \sin(2\pi y/L) + \ldots \tag{46}$$

$$M(\Theta_m) = 2 \langle P_2 \rangle \sin 2\Theta_m + \ldots \tag{47}$$

A similar description holds for nematic and smectic liquid crystals where the interaction of one molecule with its neighbours is also described by generating functions. The complicated interaction between the molecules is replaced by a mean interaction which is referred to as mean field (de Gennes 1974). The same interpretation can be applied to migrating cells where the input signal contains many details of the substrate but where these details are not all reproduced by the output signal. Therefore we can infer that the cell does not perceive all the details of the substrate, and hence, is comparable to a short-sighted man. This blurred enviroment can be compared to the mean field of liquid crystals.[1]

Nematic contact guidance: If the cell size is large in relation to the characteristic length of the substrate, the mean field is only angularly dependent. This is expressed by $(2\cos^2 \Theta_m - 1)$. How well the cell recognizes such a field is expressed by the apolar order parameter, $\langle P_2 \rangle$. This cellular situation can be compared to a nematic liquid crystal, where only the orientation of the molecules is ordered (Gruler 1988). The mean field then exhibits only angular dependence and its strength is described by the apolar order parameter, $\langle P_2 \rangle$.

1 There is a basic difference between liquid crystals and the locomotion of cells. Migrating granulocytes recognise, but do not change the physical state of their surroundings. The feedback from the cells to the substrate is zero. However, in liquid crystals, there exists a feedback from one molecule to its molecular environment. Therefore the mean field of liquid crystals has to be constructed in a self consistent way (de Gennes 1974).

Smectic contact guidance. If, on the other hand, the cell size is small in relation to the characteristic length of the substrate, then the mean field has a spatial as well as an orientational dependence. The spatial dependence is expressed by $\cos(2\pi y/L)$ and the strength of the mean field is given by the density order parameter, $\langle P_{1d}\rangle$, but no statement is made about the cellular orientation. This cellular situation can be compared to a smectic crystal where all molecules are oriented in the same direction and arranged furthermore in parallel planes (de Gennes 1974). A basic term of the smectic mean field is $\langle P_d\rangle \cos(2\pi y/L)$, with $\langle P_d\rangle$ as a density order parameter.

Knowledge of the mean field allows us to calculate many phenomenological properties of the nematic liquid crystal (i.e. calorimetric and elastic properties, entropy, etc.). We hope that it will soon be possible to predict cellular responses from such mean fields. An example where we have already been successful is for chemotaxis and galvanotaxis (Gruler and Nuccitelli 1985; Gruler 1988). Using the generating function or the mean field we have been able to calculate the apolar order parameter which describes the tactic movement as a function of the applied electric field or of the concentration gradient of the chemotactic molecules. Therefore it would be of great interest to have a detailed knowledge of mean fields for the study of cellular responses.

4. Cellular decision, anisotropic locomotion, and anisotropic surface tension

In the previous sections our results were discussed in a phenomenological way, by treating the migrating cell as a "black box". In this interpretation it was not necessary to know the interior of the "black box", that means the details behind the movement mechanism of the cell. We now want to discuss a model which describes the function of this "black box".

We have demonstrated earlier (Gruler and Bültmann 1984a, b) that the locomotion of granulocytes is governed by an internal clock and an internal program. The rhythm of the internal clock is determined by the sequential changes of cellular events. *First:* measurements of the environment. *Second:* decision about the direction of locomotion. *Third:* locomotion, etc. The repetition time for this process is the characteristic time, τ. After migrating for time τ in a certain direction, a new direction of locomotion is determined by an internal program: The cell decides basically between left or right, thereby preferring either the angle $+35°$ or $-35°$. For isotropic environments, the decision for the + and − direction is equally probable and the cell moves with the same track velocity in the + or − direction. This equivalence is disturbed if the cells are exposed to anisotropic environments, e.g. a concentration gradient of chemotactic molecules, or electric fields, or undulate surfaces. If cells are subjected to polar fields, then their migration pattern consists of a "one way" traffic movement. If an apolar field, as in undulate surfaces, is applied to the cells, than their locomotion pattern becomes a "two way" movement in opposite directions. These anisotropic environments can affect cell locomotion in three different ways: *(i)* They can destroy the symmetry between the + and − direction of migration without affecting the track velocity. *(ii)* They can alter the track velocity without affecting the cellular decision for + and − direction of migration and *(iii)* they can alter both the cellular decision and the track velocity.

We found in our experiments that cells migrate on undulate substrates with angularly dependent velocities. The question as to whether both the cellular decision process as well as the track velocity are altered may be answered by detailed analysis of the diffusion coefficients: Fürth (1920) has shown that the diffusion coefficient is proportional to the characteristic time of the motion and to the mean square of the track velocity. But the diffusion coefficient depends also on the angular change in the direction of migration. Therefore, the ratio $D(\phi)/\langle v(\phi)^2\rangle$ would be a constant independent of the angle, if the angular change of the cell were not influenced by the undulate surface. However, if the ratio $D(\phi)/\langle v(\phi)^2\rangle$ was angularly dependent then the angular change would be a function of the direction of locomotion of the migrating cell. In Fig. 10, $2D(\phi)/\langle v(\phi)^2\rangle$ is plotted vs. $2\cos^2\phi - 1$. The values are distributed along straight lines. A nearly horizontal line is evident for cells moving on an optical

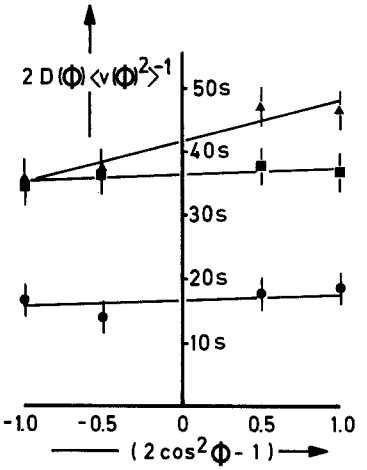

Fig. 10. The angular dependence of the ratio of the diffusion coeffient and the mean square velocity is shown for cells migrating on an optical grid ■, a scratched aluminium plate ●, and an undulated polyethylene foil ▲. A nearly horizontal line is obtained for the scratched aluminium and for the optical grid. It indicates that the cells are only guided by anisotropic surface tension

grid or a scratched aluminium plate, indicating that the anisotropy of the random walk is mainly due to the anisotropy in the track velocity. The track velocity in itself is dependent on the surface tension between a cell and the surface of the substrate and this surface tension is related to the contact angle between these two. We measured the contact angle of a drop of water on an undulate surface. The contact angle measured in the direction of the lines is larger than the contact angle measured perpendicular to the lines of the substrate. Thus our results support the observation of Weiss who showed that the contact guidance is determined by the anisotropy of the surface tension.

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