

Chemokinesis and Necrotaxis of Human Granulocytes: the Important Cellular Organelles

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Z. Naturforsch. **42c**, 1126–1134 (1987); received September 17, 1986/June 5, 1987

Cell Movement, Granulocyte, Cytokineplast, Cytochalasin B, Colchicine

The directed and non-directed locomotion of human polymorphonuclear leukocytes on a glass surface was compared to Brownian and drift motion. The average track velocity was measured under different conditions. The track velocity of colchicine treated cells was the same as control cells. However, cytochalasin B treated cells and cytokineplasts had a reduced track velocity compared with the control cells.

The non-directed locomotion was investigated by measuring the mean square displacement as a function of time. The diffusion constant, D , which quantitates the random walk process, and the characteristic time, τ , which governs the migration of the cell, was calculated. The value of the diffusion constant depended on the cell treatment: For control cells $261 \mu\text{m}^2/\text{min}$, for colchicine treated cells $145 \mu\text{m}^2/\text{min}$, for cytochalasin B treated cells $55 \mu\text{m}^2/\text{min}$, and for cytokineplasts $47 \mu\text{m}^2/\text{min}$. The characteristic time was about 40 s. The measurement showed that the non-directed locomotion can be described by the Brownian motion.

The directed locomotion was investigated by a necrotactic assay and quantitated by the McCutcheon index. This index was for control cells 0.85 ± 0.07 , for colchicine treated cells 0.8 ± 0.07 , and for cytokineplasts 0.75 ± 0.1 . The measurement showed that the directed locomotion can be described by a process which is called drift mode.

From this method of analysis it was determined that the important organelles of the cell for the directed and the non-directed locomotion are: (i) A part of the plasma membrane, (ii) the microfilaments, and (iii) an unstructured part of the cytoplasm. The microtubules of the cell are only of minor importance for the directed and the non-directed locomotion.

Introduction

The ability of a cell to detect objects in, and to respond to its environment is crucial to its existence. Among cellular abilities are the directed and non-directed movements that occur in a wide variety of both plants and animals [1]. A few examples are: The sperm of ferns is attracted by malic acid, a substance present in the egg cell. Single cells of slime molds are attracted by compounds released from bacteria since bacteria are the nutrient for these cells. Pennate diatoms are attracted by certain sugars. Bacteria, like *Escherichia coli*, respond to changes in the various chemicals in its environment such as aspartate, serine and certain sugars. Leukocytes are attracted by dying cells or by cells invaded by microorganisms. Fibroblasts, neural crest cells, epidermal cells and nerve cones migrate towards the cathode if an electric field is applied while leukocytes migrate towards

the anode. In general one can say that the symmetry of the cellular environment determines the type of movement [2]. A random movement is obtained when the cellular environment is isotropic: the cell obtains at every position and at every angle the same information. In contrast, directed movement is obtained if the cellular environment has a polar symmetry. Typical cases of this type of symmetry are the concentration gradient of chemotactic molecules (Chemotaxis), an electric field (Galvanotaxis), etc.

The molecular basis of cell movement is different for different types of cells and it is a very complex phenomenon. Nevertheless, the center of gravity of the cells can be described in the framework of the symmetry of the environment. This means the random walk activity can be described by the Einstein-Smoluchowski theory [3, 4]. Przibram [5] showed that the random movement of protozoa can be described by this theory. Fürth [6] improved the Einstein-Smoluchowski theory for a correlated walker and he showed that the random movement of infusoria can be very well described by this diffusion theory. Brokaw [7] investigated the random move-

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341-0382/87/0900-1126 \$ 01.30/0

ment of spermatozooids and found a good agreement with Fürth's theory. Gruler and Bültmann [8, 9] showed that Fürth's theory can also be applied to human granulocytes. Dahlquist *et al.* [10], Berg and Brown [11], McNab and Koshland [12] to name only a few analyzed the random walk behaviour of bacteria by using the diffusion equation which is a result of the Einstein-Smoluchowski theory.

The directed movement of cells in an environment with a polar symmetry is on the average a drift of all the cells in one direction. This type of movement can be quantified by a polar order parameter as Brokaw [7] and Gruler and Bültmann [8, 9] have shown for the movement of single cells. Expressions for the directed movement can be obtained if the symmetry of the cellular environment is considered as a basic element. (i) The polar order parameter is expressed by the Langevin function [13], as also suggested by Brokaw [7], when the cells have the possibility to migrate in the three-dimensional space. (ii) If, however, the cells have only the possibility to migrate in a two-dimensional space as *e.g.* in a flat surface, then the polar order parameter is expressed by the ratio of two modified Bessel functions (Gruler and Nuccitelli [14]). Brokaw [7] investigated the directed movement of spermatozooids exposed to an electric field. He described his experimental results with the Langevin function as predicted by the theory for the three dimensional case. But the spermatozooids can only swim in a thin layer between two glass plates so that the experiments have to be compared with theory derived for the two dimensional case. Gruler and Nuccitelli [14] investigated the galvanotactic response of fibroblasts crawling on a flat surface. The experimentally determined polar order parameter is very well described by the theoretical prediction (= ratio of two Bessel functions). The chemotaxis of granulocytes can also be described by this theory. The theoretical considerations are so general that the derived curves should describe any directed movement independent of the cell type as well as independent of the polar field which is exposed to the cells.

Not only the path of single cells but also the flux of the cell density can be used to quantify the directed locomotion. Berg and Brown [15], and Adler [16], to name only a few, analysed the directed locomotion of bacteria with the flux equation which is also based on the symmetry of the cellular environment.

Cell movements are important in embryogenesis, wound healing, malignant invasion and metastasis,

infection (Wilkinson [17]). Since the direct observation of cell locomotion inside living tissue is difficult, the movements of polymorphonuclear leukocytes were studied in tissue culture in this study. Well defined chemical and physical conditions were thus ensured. We found that infected or damaged mammalian tissues release chemotactic molecules and induce a directed locomotion of white blood cells. This *in vivo* condition was simulated *in vitro* by lysing a red blood cell by a laser flash. The locomotion of polymorphonuclear leukocytes was compared with the locomotion of inert particles. The Brownian motion of inert particles and non-directed locomotion of the cell can both be described by the laws of diffusion. While for inert particles the source for the locomotory energy is known (thermal energy), the cause of the locomoting activity for living cells is not clear. To clarify it, the cellular components which are important for the kinesis of the cell were determined. In particular we investigated the cellular components such as microtubules, microfilaments, nucleus, and the plasma membrane. By careful observation of cellular movement and by applying some basic physical principles it is possible to gain insight into the mechanism of cell motility.

Materials and Methods

Polymorphonuclear leukocyte (granulocyte) was obtained from healthy human donors. The heparinized venous blood was allowed to sediment at room temperature for about two hours in tubes tilted at about 45 degrees. The granulocytes were taken from the buffy coat. The basic control medium consisted of 80% alpha-MEM (Flow Lab.) and 20% of fetal calf serum (Flow Lab.). Either one of the following substances was added to this medium: 100 nM, 10 nM, 1 nM f-Met-Leu-Phe (N-formyl methionyl leucyl phenylalanine from Sigma), 200 μ M, 10 μ M colchicine (Calbiochem), and 0.5, 1.0, 2.5 μ g/ml cytochalasin B. Two drops of the buffy coat were suspended in 0.5 ml of the above mentioned solutions and allowed to settle for 30 min at room temperature for f-Met-Leu-Phe and cytochalasin B, and at 35 °C for colchicine. The granulocyte solution was then transferred to a microscope slide, covered by a coverslip, and the edges sealed with paraffin to form a square culture chamber. Within 4–10 min, the granulocytes settled on the surface of the microscope slide and locomoted actively. All investigations were done at 35 °C. The cells were observed

with phase contrast microscope photographed every 4 s. Usually about 20 cells were observed and photographed for about 10 min. The pictures were projected onto a screen and the centers of the cell were marked. The coordinates of these points were transferred to a small computer for further analysis.

For the preparation of the mobile fragments (cytokineplasts) the slide- and coverslip preparations were warmed to 35 °C for 10 min on a microscope stage and then transferred for 9 min to another microscope stage warmed to 45 °C. The preparation was then transferred back to the 35 °C stage on the phase contrast microscope. This treatment causes the leading edge of the cell to move forward rapidly, forming a long thin stalk of cytoplasm that often breaks to form two separate units: The cytokineplast and the cell body (Malawista and de Boisleury Chevance [18]). To produce a strong gradient of chemotactic molecules erythrocytes present in the buffy coat and transferred to the culture chamber were lysed with a ruby laser flash (694.3 nm, 3 Joules in 500 µs focused to a diameter of 5 µm) [19].

Results

Some typical tracks obtained from the time lapse cine micrographs are shown in Fig. 1. The cells of the control preparation and the cells kept in a 1 nM f-Met-Leu-Phe solution had similar tracks. However, the tracks of colchicine and cytochalasin B treated cells, which are shown in Fig. 1c and 1d, as well as the tracks of cytokineplasts shown in Fig. 1e, were different from those shown in Fig. 1a and 1b. The cytokineplasts seem *e.g.* to move for a certain time but at other times they show almost no displacement at all. But even in the “resting” state, the shape of the cytokineplast was constantly changing.

From these track records the average track velocity was measured by

$$v_c = M^{-1} \sum_{j=1}^M (\bar{v}_c)_j \quad (1)$$

where $(\bar{v}_c)_j$ is the average track velocity of the j -cell. M is the total number of cells.

$$(\bar{v}_c)_j = (N_j - 1)^{-1} \sum_{i=2}^{N_j} \sqrt{((x_i - x_{i-1}))^2 + (y_i - y_{i-1})^2 (t_i - t_{i-1})^{-1}}. \quad (2)$$

The coordinates x_i and y_i were determined at N_j different time t_i . The results are summarized in Table I: The average track velocity v_c was higher in the tripeptide f-Met-Leu-Phe treated cells compared to control cells. (ii) The average track velocity of cells in 10 µM colchicine was similar to that of control cells. (iii) The average track velocity of cells in 1 µg/ml cytochalasin B and the velocity of the cytokineplast, was greatly reduced with respect to the control cells.

The locomotion of a cell in an isotropic distribution of chemokinetic molecules is a random walk process which is described by the mean square displacement $\frac{1}{2} (\overline{\Delta x^2} + \overline{\Delta y^2})$ as a function of time t (Gruler [20]). From the track records the mean square displacement was measured by calculating the travelled distance between the time t_i and t_1 :

$$(\Delta x_i)^2 + (\Delta y_i)^2 = (x_i - x_1)^2 + (y_i - y_1)^2. \quad (3)$$

This expression holds for one cell. The average over M cells gives the mean square displacement:

$$\frac{1}{2} (\overline{\Delta x_i^2} + \overline{\Delta y_i^2}) = \frac{1}{2M} \cdot \sum_{i=1}^M (\Delta x_i^2 + \Delta y_i^2). \quad (4)$$

The mean square displacement vs. time is shown in Fig. 2 for the different experimental conditions.

Table I. Track velocity, diffusion constant, characteristic time, and p-value.

	Control cells	f-Met-Leu-Phe 1 nM	10 nM	100 nM	Colchicine 10 µM	Cytochalasin B 1 µg/ml	Cytokineplast
average track velocity v_c [µm min ⁻¹]	21.4 ± 5	26.5 ± 5	24.9 ± 5	23.6 ± 5	21.8 ± 5	9.8 ± 3	12.6 ± 4
diffusion constant D D [µm ² min ⁻¹]	261 ± 20	390 ± 37	515 ± 37	356 ± 30	145 ± 15	55 ± 15	47 ± 3
characteristic time τ [s]	42 ± 5	40 ± 5	55 ± 5	60 ± 6	40 ± 5	43 ± 5	25 ± 4
calculated p-value $p = 4 \cdot D \cdot v_c^{-2} \cdot \tau^{-1}$	3.2 ± 0.8	3.3 ± 0.7	3.6 ± 0.7	2.5 ± 0.6	1.8 ± 0.5	3.2 ± 1.5	2.8 ± 0.9

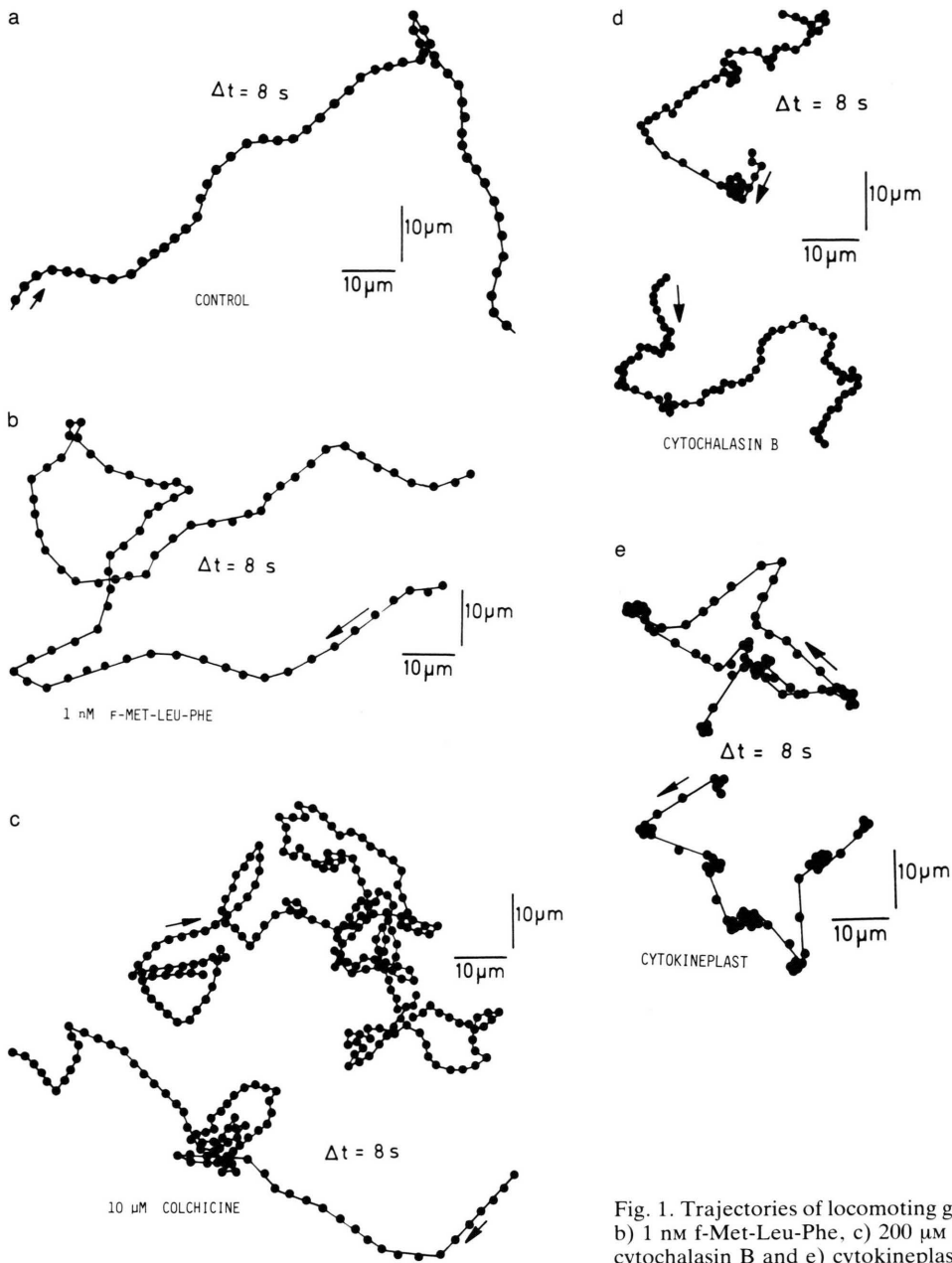


Fig. 1. Trajectories of locomoting granulocytes. a) Control, b) 1 nM f-Met-Leu-Phe, c) 200 μ M colchicine, d) 2.5 μ g/ml cytochalasin B and e) cytokineplast.

The basis for the evaluation of these data is the following: Newton's laws applied to the center of mass of the locomoting cell yield the following equations

$$m \frac{d^2x}{dt^2} = F_x(t); m \frac{d^2y}{dt^2} = F_y(t) \quad (5)$$

where m is the effective mass of the cell. $\vec{F}(t)$ ($= (F_x, F_y)$) describes the action of the locomotive machinery of the cell. The aim is to get some information about this unknown function $\vec{F}(t)$. Due to the fluctuation in the processes involved in locomotion one expects that the force also fluctuates in time.

However, this force $\vec{F}(t)$ is not totally undetermined because the locomotory machinery of the cell creates the speed v_x and v_y of the cell. This yields

$$F_x(t) = -B^{-1} \cdot v_x + f_x(t); F_y(t) = -B^{-1} \cdot v_y + f_y(t) \quad (6)$$

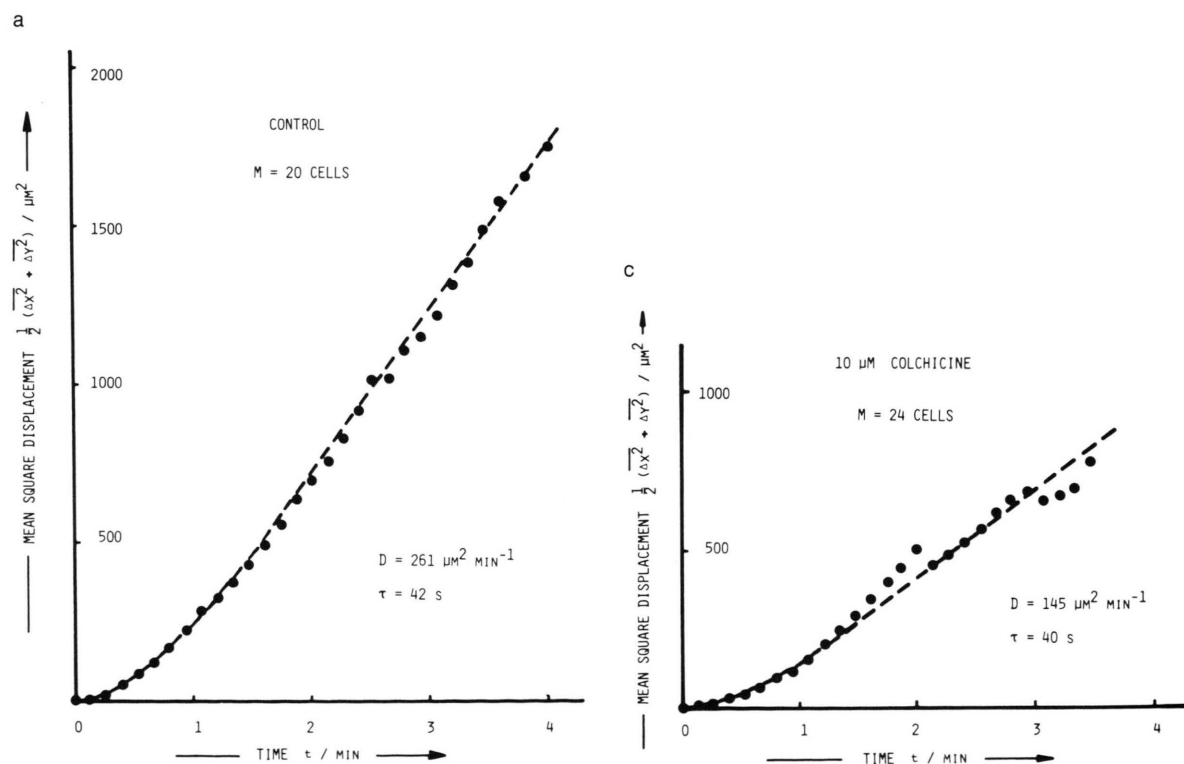
where B is a constant. The first term in these equations describes the average force and the second term describes the stochastic part of the force whose time average is zero. The differential equation obtained from Eqns. (5) and (6) is known as the Langevin equation (Reif [7]). Its solution is

$$\overline{\Delta x^2} = \overline{\Delta y^2} = 2 D (t - \tau (1 - e^{-t/\tau})) \quad (7)$$

where $\overline{\Delta x^2}$ and $\overline{\Delta y^2}$ are the mean square displacements of the cell, τ is the characteristic time ($= B \cdot m$), and D the diffusion constant ($= \tau \cdot v_x^2 = \tau \cdot v_y^2$) of the locomoting cell. Over long durations, one gets

$$\overline{\Delta x^2} = \overline{\Delta y^2} = 2 D (t - \tau) \quad t \gg \tau. \quad (8)$$

The mean square displacement vs. time is shown in Fig. 2 for different experimental conditions. Eqn. (7) describes the graph of the experimental points. The values of the diffusion constant D , and characteristic time τ , used to fit Eqn. (7) to the experimental data



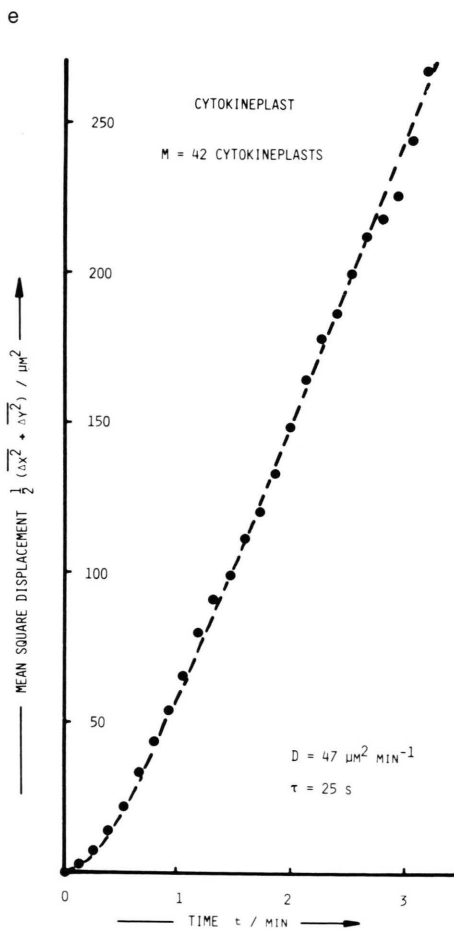
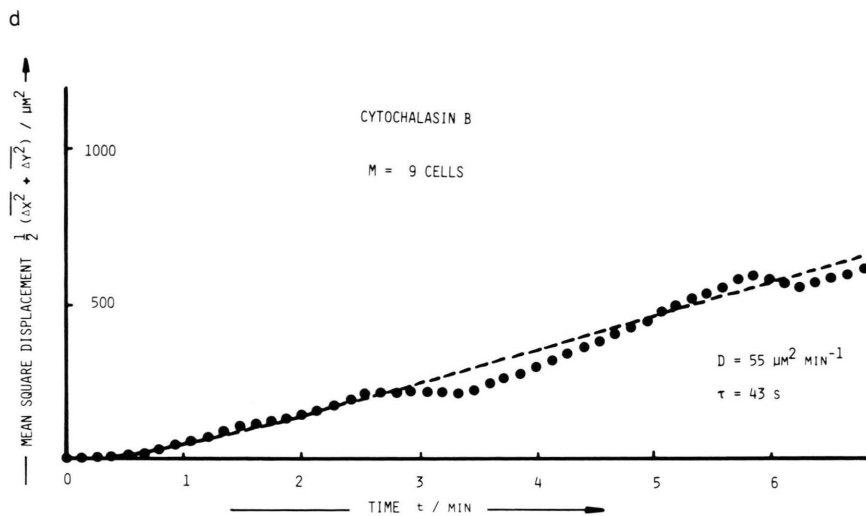


Fig. 2. Mean square displacement $\frac{1}{2} (\overline{\Delta x^2} + \overline{\Delta y^2})$ as a function of time. M is the number of observed cells. The circles are experimental points. The dashed line was obtained by fitting the experimental points to eq. 7. a) Control, b) 1 nM f-Met-Leu-Phe, c) 200 μM colchicine, d) 2.5 $\mu\text{g/ml}$ cytochalasin B and e) cytokineplast.

is shown in Table I: Compared to control cells the diffusion constant is higher for cells treated with the tripeptide f-Met-Leu-Phe. The diffusion constant of locomoting cells is decreased by colchicine and greatly reduced by cytochalasin B. In addition cytokine-plasts also have lower diffusion constants. The characteristic time for control cells, cells in colchicine, 1 nM f-Met-Leu-Phe, cytochalasin B is about 40 s. Cells in higher concentrations of f-Met-Leu-Phe (> 10 nM) have a larger characteristic time constant. The characteristic time of cytokineplasts is about 25 s.

It is clear that due to the stochastic nature of the fluctuating part of the force one can not give an analytic expression for it. However, the time average of the square of $f_x(t)$ and $f_y(t)$ is positive and its value is proportional to the value of the diffusion constant.

The directed movement of cells in a gradient of chemotactic molecules was investigated by means of necrotactic assay. The advantage of this technique is that the cells can be investigated in an isotropic distribution of chemokinetic molecules and then the same cells can be exposed to an anisotropic distribution of chemotactic molecules. The disadvantage is that there is up to now no way to measure or quantitate the chemotactic gradient.

At time t_0 an erythrocyte in the culture chamber was lysed by Laser flash. The movements of cells was recorded within a distance of about 50 μm from the lysed cell for about 3 min. From the record of the cell movement the McCutcheon index was determined (Gruler [20])

$$\overline{P}_1 = \frac{1}{M} \sum_M (r(t_0) - r(t)) \Delta s^{-1}. \quad (9)$$

During the time, $t - t_0$, the cell moved the distance, $r(t_0) - r(t)$, towards the necrotactic source ($r=0$). Δs is the distance along the track. The McCutcheon index for control cells, colchicine treated cells, and cytokineplasts was 0.85 ± 0.05 , 0.8 ± 0.07 , and 0.77 ± 0.1 respectively.

Discussion

A. Non-directed locomotion

The microscopic properties of the locomotory machinery influence the macroscopic properties of the non-directed cell locomotion. Perturbation of cellular organelles can reveal the elements of the

locomotory machinery. We are particularly interested in the importance of the cell membrane, microtubules, and microfilaments in cell locomotion.

As a first step the locomotory activity of a cell can be quantitated by the mean track velocity, v_c . This activity of the cell is stimulated by its chemical surrounding. The track velocity of cells in 100% human plasma is $23.9 \pm 5 \mu\text{m}/\text{min}$. The average track velocity measured in a solution containing only 20% fetal calf serum is decreased to $21.4 \pm 5 \mu\text{m}/\text{min}$. This effect disappears again with increasing concentration of f-Met-Leu-Phe. This means that the granulocytes surrounded by 100% human plasma locomote in average with its maximum speed.

The mean track velocity was not affected by colchicine. As colchicine disrupts the microtubules, this suggests that microtubules are not important for the locomotory activity of the cell. This conclusion is also confirmed by the locomoting cytokineplasts which show locomotory activity though they do not contain microtubules (Malavista and de Boisleury Chevance [18]). However, microfilaments are involved in cell locomotion as the mean track velocity of granulocytes was reduced when cells were treated with cytochalasin B which is known to disrupt microfilaments [21].

As the second step the characteristic time was determined from the mean square displacement. The characteristic time was the same for the control cells for the cytochalasin B and colchicine treated cells, and also cells in 1 nM f-Met-Leu-Phe. However, cells in higher concentrations of f-Met-Leu-Phe had larger characteristic times then the control cells. The characteristic time gives the interval between two successive changes in direction of movement. Therefore the characteristic time can be taken as a measure of the internal clock of the migrating cell.

Finally the random walk activity of cells was quantitated by the value of the diffusion constant. The diffusion constant was derived from the slope of the mean square displacement vs. time. The value of the diffusion constant changed due to different treatments of the cells. To understand why these values differ one has to understand the relation between the various factors that can influence the value of the diffusion constant. The relation between these various factors is described by the random walk model: The cell locomotes between two successive changes in direction of direction with a constant track velocity v_c . The change in angular direction of migration is

then $\pm \alpha$. From this one obtains the diffusion coefficient as [22]

$$D = \frac{v_c^2 \cdot \tau}{4} \cdot p \quad (10)$$

$$\text{with } p = \frac{1 + \cos \alpha}{1 - \cos \alpha} \quad (11)$$

The diffusion constant depends on the track velocity, the characteristic time, and the p -value which characterizes the amount of change in direction of movement. We can concentrate our discussion to the p -value since the characteristic time and the track velocity were already discussed. The p -value derived from D , v_c , and τ is shown in Table I. For 1 nM f-Met-Leu-Phe, the p -value is comparable with that of the control cells. At this concentration only the track velocity is influenced by this tripeptide. The random walk activity of neutrophil polymorphonuclear leukocytes is decreased in the case of colchicine. One finds that the p -value ($= 1.8$) is smaller than that of control cells ($= 3.2$). Therefore colchicine increases the average angle between old and new direction. The random walk activity of cytokineplasts is small compared with that of control cells. This results partially from the low track velocity, but also from a significantly lower p -values compared with control cells. One could say that cells without microtubules (colchicine treated cells, cytokineplasts) make in average a larger angular changes between old and new direction of migration than cells with microtubules.

Cytochalasin B reduces the random walk activity of the cell. This reduction can be simply explained by the reduction of the track velocity. There is no effect on the p -value. This means that the density of the microfilaments is not important for the choice of the new moving direction.

Directed locomotion

The locomoting cells in a concentration gradient of chemotactic molecules drift on the average parallel to the gradient. This type of movement can be characterized by (i) the average drift velocity, $\overline{v_{||}}$, (ii) the chemotropism index, $\overline{v_{||}}/\overline{v_c}$, the McCutcheon index ($=$ the ratio of the distance moved parallel to the concentration gradient to the distance moved along the trajectory), and (iv) the polar order parameter, $\overline{P_1}$. The locomoting cells are oriented in the concentration gradient so that the average of the first Legendre polynom $\overline{P_1(\varphi)} = \overline{\cos \varphi}$ can be deter-

mined. According to Gruler [20], and Gruler and Bültmann [23] there is no correlation (or only a weak one) between the track velocity and the change in moving direction. This means there is no difference between the polar order parameter $\overline{P_1}$, the chemotropism index and the McCutcheon index.

The McCutcheon index of human granulocytes determined in a necrotactic assay is 0.85 ± 0.05 . The polar order parameter determined from measurements in a Zigmond chamber with 10 nM/mm f-Met-Leu-Phe is 0.8 ± 0.05 . This agreement demonstrates that the necrotactic assay can be compared with the Zigmond chamber.

The organelles which are important for the non-directed cell locomotion are also important for the directed cell locomotion. The McCutcheon index of colchicine treated cells is nearly unchanged compared with that of control cells which means that the microtubules are not important for the directed locomotion. Though the mean track velocity of cytokineplasts is lower than control cells, the McCutcheon index is not significantly different. This indicates that microtubules play no or only a minor role in the directed cell movement, a conclusion consistent with the fact that cytokineplasts do not have microtubules.

The characteristic time plays an important role in the directed locomotion of granulocytes. This is best demonstrated in an experiment where two erythrocytes were lysed in the culture chamber. When the first erythrocyte in the center of the viewing field was lysed, it created a necrotactic gradient. After a characteristic time ($= 40$ s) all the cells in the field drifted towards the necrotactic source. Then another necrotactic source was created at the border of the viewing field. Granulocytes which are close to the new necrotactic source still move towards the first necrotactic source located in the center of the old viewing field for up to 20 s before migrating towards the second necrotactic source.

Conclusion

The machinery which creates the cell locomotion contains only a few elements. The plasma membrane, the microfilaments, and the unstructured cytoplasm as seen by light microscope. The locomotive state is governed by an internal clock of the cell. One believes that this clock has its origin in a chemical oscillating reaction.

Acknowledgements

We would particularly like to thank Prof. M. Bessis for fruitful and stimulating discussions. The work

was supported by "Fond der chemischen Industrie" and by Nato travel grant.

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