Analysis of the Directed and Nondirected Movement of Human Granulocytes: Influence of Temperature and ECHO 9 Virus on N-formylmethionylleucylphenylalanine-induced Chemokinesis and Chemotaxis

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ABSTRACT  The directed movement of human polymorphonuclear leukocytes (PMN) in a plane (Zigmond chamber assay) is described by a statistical model. We demonstrate that (a) the movement of a single cell is a superposition of a directed and a random movement, and (b) the degree of orientation, $P_1$, of moving cells in a chemotactic gradient can be determined either by the time average of a single cell or by the average of movement of multiple cells at a fixed time (Ergoden hypothesis). However, an homogeneous cell population is a necessary condition. $P_1$, which is identical with the McCutcheon index, is derived from the measured angular distribution function of moving cells. The statistical model allows one to distinguish between chemotaxis and chemokinesis. Applying this model to the temperature-dependent changes of cell movement, we found that $P_1 = 0.82$ (37°C) decreased to $P_1 = 0.4$ (22°C). The average speed of moving cells exhibits a very strong temperature-dependent variation from 30 μm/min (37°C) to 5 μm/min (22°C), indicating a different temperature dependence of chemotaxis and chemokinesis. At a fixed temperature (37°C) the stability of the chemotactic gradient can also be checked by the angular distribution function. In addition, this model was applied to investigate the enteric cytopathogenic human orphan, strain 9 (ECHO 9) virus-induced disturbances of cell movement. We found: (a) The average speed of cell movement is not affected by the virus. (b) The degree of orientation is not affected for virus doses below a critical virus dose, $a_0$ (virus/PMN = 0.8:1). (c) The degree of orientation above this critical value exhibits a time- and virus-dose-dependence. (d) At a fixed viral dose, the time-dependent decrease of $P_1$ is described by an exponential law (virus/PMN = 5:1, the characteristic time is 110 min). (e) This characteristic time investigated as a function of viral dose results in a logarithmic law analogous with the Weber-Fechner law. These findings indicate that only chemotactic and not chemokinetic response is disturbed by ECHO 9 virus.

The ability of some cells or primitive organisms to direct their movement along a chemical gradient seems to be a general phenomenon (1). Lower organisms, e.g., bacteria or slime molds, exhibit movements directed to nutrients. Following infection or other damage of mammalian tissue, chemotactic factors are released and induce movement of polymorphonuclear leukocytes (PMN) to the site of injury. During this process these cells can orient their locomotion according to a chemical gradient, i.e., exhibit chemotaxis (2). This chemotactic reactivity has been tested in vitro using different assays. One of them was designed by Zigmond (2) and will be employed in our investigations. Beside these different in vitro assays, there exist some statistical models of cell movement that are based on the diffusion equation (3–6). The purpose of this paper is to demonstrate a general mathematical model of directed cell movement, and to show the relationship between directed and nondirected movement, as well as the relationship between the movement of an individual cell and that of a population of...
cells. Our model is a mathematical description of cell or particle movement and thus, necessarily, is not restricted to human PMN. The practical importance of our investigations lies in a detailed analysis of the experimental conditions of the Zigmond chamber assay to demonstrate that preincubation of human PMN with enteric cytopathogenic human orphan virus, type 9, strain A. Barty (ECHO 9 A.B.) results in disturbances of cellular response to chemotactic agents (zymosan-activated serum, N-formylmethionylleucylphenylalanine). This technique, however, has certain limitations regarding the discrimination between chemokinesis and chemotaxis (8). Therefore in the present investigations, the Zigmond chamber assay and our mathematical model of cell movement was applied for further analysis of this virus-induced phenomenon. We will show that only chemotactic but not chemokinetic reactivity of virus-treated PMN will be inhibited.

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MATERIALS AND METHODS

Granulocytes

Leukocytes were separated from heparinized venous blood of healthy human blood donors by means of dextran sedimentation or Ficoll-Hypaque density gradient as previously described (8). After osmotic lysis of the remaining red blood cells and readjustment of osmotic strength, the leukocytes were washed three times, resuspended in Hanks' solution supplemented with 1% gelatine (Hanks' gel), and adjusted to $5 \times 10^5$ PMN/ml as stock suspension.

Virus

ECHO 9 A. B. was originally derived from a patient with aseptic meningitis (10). As previously reported, this strain was propagated in primary cell cultures of trypsinized kidney tissue from African green monkeys (Flow Laboratories, Inc., McLean, VA); for infectivity titrations (plaque-forming units), a continuous cell line derived from African green monkey renal tissue was used (7).

To study the interaction of ECHO 9 A. B. virus with granulocyte function, 2 x $10^6$ PMN/ml were preincubated ($t_0$) with different high doses of virus (2 x $10^6$, 1 x $10^7$, 4 x $10^7$, and 1 x $10^8$ pfu) for 15 min at 37°C in a shaking water bath. Some preparations of 2 x $10^6$ PMN/ml were preincubated with 1 x $10^7$ pfu ECHO 9 A. B. virus for 60 min at 37°C. Subsequently, the cells were washed 3 times and resuspended to the original volume. The virus suspension was sonified for 5 s before incubation with PMN.

Chemotactic Factor

The chemotactic factor used was N-formylmethionylleucylphenylalanine (FMLP) supplied by Vega-Biochemicals (Div. of Vega Biotechnologies, Inc., Tucson, AZ). Stock solutions were prepared with dimethyl sulfoxide and stored at $-20°C$. Assays of FMLP were carried out in Hanks' gel in concentrations of 10$^{-6}$, 10$^{-7}$, 10$^{-6}$, and 10$^{-9}$ M.

Chemotaxis Assay

The chemotactic chambers were set up as described by Zigmond (2). Briefly, 50 μl of the cell suspension were pipetted on a round (22 mm q) No. 1 glass coverslip. The cells attached to the glass surface to form a monolayer during incubation for 15 min in a 37°C humid incubator gassed with 5% CO₂. The coverslip with adherent PMN was rinsed twice with Hanks' gel, then inverted, placed on the Zigmond chamber, and secured with brass clips. Excess buffer was removed by gentle suction; the wells were filled with 100 μl of FMLP in one well, whereas the other contained Hanks' gel only. Both wells were sealed by paraffin wax to avoid loss of medium by evaporation. The loaded Zigmond chamber was placed on the heated (37°C) object stage of a Zeiss photomicroscope equipped with an interference optical system (x40).

Mathematical Model of Directed Cell Movement

MODEL OF INDIVIDUAL CELL MOVEMENT: We have the following problem: A granulocyte adhering to a plane surface has the intrinsic possibility of motility. This is demonstrated by the fact that irregular cytoplasmic propulsions of protruberances are always present without change in the original adhering position (Fig. 1a). Exposure of the cell to a chemotactic stimulus results in active movement accompanied by characteristic cell shape changes and by change of the starting position (Fig. 1, b and c). The movement of the center of the granulocyte mass (S) in a plane (Fig. 2) can be described by the following vector:

$$v = \mathbf{x}_0 \cdot V_c \cdot \cos \varphi + \mathbf{y} \cdot V_c \cdot \sin \varphi.$$  

where \( \mathbf{x}_0 \) and \( \mathbf{y} \) are cartesian unit vectors in the plane. The speed of the granulocyte is described by the track velocity \( V_c \). The direction of movement is described by the angle \( \varphi \). The path of the migrating cell is described by the time dependence of the angle \( \varphi(t) \). It is assumed that \( V_c \) is constant in time at standardized experimental conditions. At time zero, the cell is at the starting point. After the time \( t \), the cell migrated in both \( x \) and \( y \) directions.

$$x = \int V_c \cdot \cos \varphi(t) \cdot dt,$n\text{.}$$

$$y = \int V_c \cdot \sin \varphi(t) \cdot dt.$n\text{.}$$

FIGURE 1 Cytological changes of granulocytes in a Zigmond chamber. (a) Random mobility of cells not exposed to chemotactic agents. (b) Chemokinetic movement induced by a homogeneous environment of 10$^{-8}$ M FMLP. (c) Chemotactic response of PMN exposed to a chemotactic gradient of 10$^{-8}$ M FMLP/mm (fat arrow). Angle \( \varphi \) is defined by vectors of chemotactic gradient and velocity \( v \) of granulocyte.
by the vectors of the track velocity and the chemotactic gradient. The velocity parallel and perpendicular to the chemotactic gradient is $v_x \cdot \cos \phi$ and $v_x \cdot \sin \phi$, respectively.

These equations can be rewritten as

$$v = (V_1 \cdot \cos \phi(t)), \quad (4)$$

$$y = (V_1 \cdot \sin \phi(t)), \quad (5)$$

$(\cos \phi)$ and $(\sin \phi)$ are the time average of $\cos \phi(t)$ and $\sin \phi(t)$.

$$(\cos \phi) = \frac{1}{t} \int_{t_0}^{t} \cos \phi(t) \, dt, \quad (6)$$

$$(\sin \phi) = \frac{1}{t} \int_{t_0}^{t} \sin \phi(t) \, dt. \quad (7)$$

For a nondirected movement, $(\cos \phi)$ and $(\sin \phi)$ are zero. This means that the migrating cells move in a random fashion around the starting position. This type of movement is referred to as chemokinesis or random mobility (8, 9) of the granulocyte (Fig. 1a). In our case the random mobility can be characterized by the track velocity, $V_t - V_i$, which is dependent on environmental conditions such as the strength of chemotactic agent, pH, and temperature (8, 12). In a directed movement, $(\cos \phi)$ and $(\sin \phi)$ are not equal to zero owing to cell orientation accompanied by characteristic changes of cell shape (Fig. 1c). A preferred direction can be achieved by introducing a chemical gradient of a chemotactic material into the experimental system (Fig. 2). This kind of directed cell movement is referred to as chemotaxis (3, 8, 9). If this preferred direction is parallel to the $x$ direction of the plane (chemotactic gradient), then

$$\langle \cos \phi \rangle \neq 0, \quad (8)$$

$$\langle \sin \phi \rangle = 0. \quad (9)$$

Since $V_1 \cdot \langle \cos \phi \rangle$ describes the strength of the directed cell movement, we will use $(\cos \phi)$ to refer to the degree of cell orientation $P_1$. This definition is used in analogy with a physical system describing the orientation of molecules in a field (e.g., the orientation of elementary magnets in a magnetic field or the orientation of molecular electric dipoles in an electric field).

$$P_1 = \langle \cos \phi \rangle = \frac{1}{\tau} \int_{t_0}^{t} \cos \phi(t) \, dt \quad (10)$$

The maximal degree of orientation can be 1. In this situation the cell moves in a straight line parallel to the preferred direction. In the case of $0 < P_1 < 1$, the cell moves in a snakelike path in the direction of the chemotactic gradient, as shown in Fig. 2. The average speed in the direction of the chemotactic gradient, $V$, defines the biological function (chemotaxis):

$$V_t = V_1 \cdot P_1. \quad (11)$$

Based on this general mathematical description of cell movement, it is possible to measure quantitatively the chemotactic response of an individual granulocyte in a Zigrnond chamber exposed to a chemotactic gradient. The speed $V_t$ and the degree of orientation $P_1$ can be evaluated from a series of pictures taken consecutively and photographed during a period of time after the chemotactic gradient has been stabilized (Fig. 3).

$$V_t = \frac{1}{N_t - 1} \frac{\sum \Delta x_i + \Delta y_i}{\Delta t}, \quad (12)$$

The degree of orientation is

$$P_t = \frac{1}{N_t - 1} \frac{\sum \Delta x}{\sqrt{\sum (\Delta x_i + \Delta y_i)^2}}, \quad (13)$$

$N_t$ is the total number of pictures.
RESULTS

Comparison between Single Cell Movement and the Behavior of a Population of PMN in a Chemotactic Gradient

For investigation of the track velocity, $V_c$, and the degree of orientation, $P_{time}$, of individual moving PMN ($n = 348$ cells/10 experiments), the granulocytes were exposed to $10^{-8}$ M FMLP/mm. In the established chemotactic gradient (15-20 min after loading the Zigmond chamber) the cells (20-40 PMN/time and experiment) were followed by a series of single micrographs taken every 20 s for 5 min. All cells in the high power microscopic field were investigated independent of their initial shapes.

As shown in Fig. 4, the real path of an individual granulocyte results in a snakelike movement parallel to the chemotactic gradient. The track velocity, $V_c$, of this granulocyte was $30 \mu$m/min. The average track velocity of the other granulocytes was $30 \pm 5 \mu$m/min. The distribution function, $N(V_c)$, resulted in a single, broadly distributed gaussian curve ($\sigma = 5 \mu$m/min). This indicates that the track velocity fluctuates during the measured time but we have no indication from our experimental data that subpopulations of moving cells are present.

From the same PMN used for the determination of the track velocity, the degree of orientation, $P_{time}$, was derived. A small period of the snakelike path of the granulocyte shown in Fig. 4 is schematically drawn in Fig. 3. The local orientation of the PMN during this path at different times is described by the angle $\gamma_0$ (see Fig. 4). Therefore we can determine the angular distribution function $N(\gamma_0)$ of such a snakelike path, which is shown on the right side of Fig. 4. As expected for a snakelike path, the distribution, $N(\gamma_0)$, exhibits a maximum at $0^\circ$. However, the movement of the cell is not a straight line parallel to the chemotactic gradient. Therefore the distribution function $N(\gamma_0)$ reveals variations of cell orientation at different times. The degree of orientation of this snakelike path (left side of Fig. 4) is $P_{time} = 0.8$ according to Eq. 13. The average degree of orientation, $P_{time}$, of the other moving single cells is $0.8$ with a standard deviation of $\pm 0.05$. Analysis of the distribution function of $N(\gamma_0)$ reveals a narrow-based gaussian curve ($\sigma = 0.05$) without evidence for subpopulations.

Next, the degree of orientation of a population of cells, $P_{pop}$, at a fixed time was calculated. For this purpose the angular distribution function $N(\gamma_0)$ of 80-100 PMN was determined from 4 to 6 photographic pictures/time taken at $t_m = 3, 15, 30$, and 45 min after loading the Zigmond chamber. Fig. 5 a summarizes the angular distribution of a cell population of a representative experiment in which granulocytes have been exposed to a $10^{-8}$ M/mm gradient of FMLP for 45 min. The distribution functions, $N(\gamma_0)$, peak around $0^\circ$. This indicates that most cells are oriented parallel to the chemotactic gradient (Fig. 1c). The degree of orientation for this experiment was $P_{pop} = 0.85$. A series of further experiments ($n = 5$) under identical conditions revealed that $P_{pop}$ was reproducible within only small statistical variations with an average of $P_{pop} = 0.82 \pm 0.05$ for 520 PMN ($t_m = 45$ min). The distribution function, $N(P_{pop})$, is described by a narrow-based ($\sigma = 0.05$) gaussian curve without evidence for subpopulations. As expected according to Eq. 14, the degree of orientation, $P_{pop}$, determined for a population of PMN is identical within the same statistical range with the degree of orientation, $P_{time}$, of an individual moving granulocyte.

Influence of Temperature on FMLP-induced Cell Movement

As already mentioned, various factors influence cell movement and orientation (8, 9). Relatively little information is available from the literature about the effect of temperature (12). Therefore, we exposed PMN to $10^{-8}$ M FMLP/mm at $37^\circ$C and at room temperature ($22^\circ$C) and took micrographs of 80-120 PMN/time at 3, 15, 30, and 45 min after loading the Zigmond chamber. The results are shown in Fig. 6. During the first 10 min after the beginning of the experiments ($n = 3$/temperature), the chemotactic gradient is not established (dashed line in Fig. 6) according to Zigmond (2). As expected, $P_{pop}$ scattered around $0^\circ$ for both temperatures. After the chemotactic gradient stabilized, $P_{pop}$ exhibited temperature-dependent marked variations: (a) Cells ($n = 285$ PMN) exposed to FMLP at $37^\circ$C for 45 min approach the saturation value of $P_{pop} = 0.82 \pm 0.05$. (b) For PMN ($n = 326$) exposed to the same chemotactic gradient at $22^\circ$C for 45 min, the saturation value $P_{pop}$ reached only $0.4 \pm 0.05$. The angular distribution function, $N(\gamma_0)$, and the distribution function, $N(P_{pop})$ for both temperatures revealed a narrow-based gaussian curve ($\sigma = 0.05$), respectively, without evidence for subpopulations.

In three further experiments the track velocity, $V_c$, of PMN ($n = 120$/temperature) was investigated by a series of single micrographs taken every 20 s for 5 min in the established chemotactic gradient ($t_m = 35-45$ min after loading the Zig-
distribution functions $N(V_{\Delta \gamma_{c}})$ and $N(V_{\Delta \gamma_{c}})$ disclosed a single gaussian curve respectively, without indication of subpopulations.

From the same cells used for the determination of the track velocity, $V_{o}$, the degree of orientation, $P_{time}$, was derived. For both temperatures, the average values of $P_{time,37^\circ C} = 0.8$ and $P_{time,22^\circ C} = 0.39$ equal the experimental data of $P_{pop}$ at $37^\circ C$ and $22^\circ C$ within the same statistical range ($\pm 0.05$).

Influence of ECHO 9 A. Virus on FMLP-induced Cell Movement

On the basis of our recent observations of ECHO 9 A. B. virus-induced diminished cellular chemotactic response of PMN in the Boyden chamber (7), we would now like to discriminate between disturbances of chemotactic and/or chemokinetic behavior of granulocytes. This can be done by evaluating the data of Eq. 11. We determined the degree of orientation, $P_{pop}$, of virus-treated cells at different times at $37^\circ C$.

In pilot experiments ($n = 3$) the angular distribution functions $N(\phi)$ of a population ($n = 80-100$ cells) of control granulocytes and virus-treated PMN (granulocyte/virus = 1:20, $t_{o} = 15$ min) both exposed to $10^{-8}$ M FMLP/mm for $t_{o} = 45$ min have been evaluated. As already shown in Fig. 5a, the distribution functions $N(\phi)$ of control granulocytes peak around $0^\circ$. This indicates that most of the cells are oriented parallel to the chemotactic gradient (Fig. 1c). The analogous distribution of virus-treated PMN has a less pronounced peak at an angle $-0^\circ$ (Fig. 5b) exhibiting a cell orientation similar to Fig. 1b. Using Eq. 17 for these experiments we obtained a degree of orientation for control granulocytes of $P_{pop} = 0.76 \pm 0.05$ and of $P_{pop} = 0.26 \pm 0.05$ for virus-treated cells. The track velocity, $V_{o}$, derived from a series of micrographs taken every 20 s over 5 min (20–30 min after start of the experiment in the Zigmond chamber), was also measured for both experimental assays. The average track velocity of $n = 236$ cells was $29.6 \pm 3.2 \mu m/min$. The distribution function, $N(V_{o})$, for both experimental groups disclosed a single gaussian curve without evidence for subpopulations.

In the following experiments ($n = 5$/viral dose) the influence of viral dose on the degree of orientation of FMLP-induced cell orientation was investigated. Granulocytes were preincubated with different doses of ECHO 9 A. B. virus (granulocyte/virus ratio ranging from 1:1 to 1:50) for $t_{o} = 15$ min and then exposed to a chemotactic gradient ($10^{-8}$ M FMLP/mm). Photographs of cell populations (80–120 PMN/time and experiment) were taken $t_{m} = 3, 15, 30,$ and 45 min after starting the chemotaxis assay. As shown in Fig. 7 the degree of orientation, $P_{pop}$, declined in time with rise in the dose of virus from $P_{t} = 0.8 \pm 0.05$ of control cells to $P_{t} = 0.15 \pm 0.05$ of PMN preincubated with virus (granulocyte/virus = 1:50) 45 min after starting the experiment in the Zigmond chamber. Fig. 8a summarizes the viral dose-dependent changes of the degree of orientation at a fixed time $t_{o} = 15$ min, $t_{m} = 45$ min. Again, a viral dose-dependent decline of the degree of orientation, $P_{pop}$, occurs, indicating a change in cellular behavior from chemotactic to chemokinetic response (random mobility).

Next, the degree of orientation, $P_{pop}$, is investigated as a function of time and viral dose on the degree of orientation, $P_{t}$, of virus-treated PMN exposed to $10^{-8}$ M FMLP/mm. The preincubation time, $t_{p}$, is 15 min. The vertical dashed line describes the end of the phase of building up the chemotactic gradient. •, control granulocytes with its extrapolated value for virus-treated PMN at the time $-t_{p}$ represented by the point, $X$, granulocyte/virus ratio: $\square$, 1:1; $\Delta$, 1:5; $+$, 1:20; $\bigcirc$, 1:50.
function of time at a given viral dose. Therefore, PMN were
preincubated for \( t_{p1} = 15 \) min \((n = \text{four experiments})\) and \( t_{p2} = 60 \) min \((n = \text{four experiments})\) with ECHO 9 A. B. virus 
(granulocyte/virus = 1:5) and then exposed to a chemotactic 
gradient \(10^{-8} \text{ M FMLP/mm}\). The degree of orientation, \( P_{1,\text{pop}} \), 
of both cell preparations \((n = 786 \text{ PMN})\) before virus-treatment 
is \( P_1 = 0.8 \pm 0.05 \) (Fig. 8b), which is the value of control cells, 
\( P_{1,0} \). The degree of orientation for a population of PMN 
\((n = 396)\) preincubated for 15 min \((t_{p1})\) was measured for 45 min 
in the Zigmond chamber \((t_{\text{ml}})\). In accordance, \( t_{\text{ml}} \) describes the 
degree of orientation of PMN \((n = 396)\) preincubated for 60 
min \((t_{p2})\). The degree of orientation decreases with increasing 
duration of virus-PMN interaction and can be described by an 
exponential law:

\[
P_1(t) = P_1^0 \cdot e^{-t/\tau}.
\]

The exponential function (dashed line in Fig. 8b) is obtained 
by a "least square fit" procedure with a characteristic decay 
time, \( \tau \), of 110 ± 5 min. This means that the degree of 
orientation (chemotaxis) is divided by a half-life period of 72 
± 4 min \((t_{1/2} = \ln 2)\).

So far, we analyzed the data at a fixed time and at a fixed 
protein dose. However, it is possible to analyze the data in such 
a way that the results lead to a better interpretation of the 
protein-granulocyte interaction. Therefore, the characteristic 
time, \( \tau \), was investigated as a function of virus dose, \( a \). The 
results are shown in Fig. 9. In this special plot, the data follow 
a straight line described by

\[
\left( \frac{\tau_0}{\tau} \right)^2 = \ln \left( \frac{a}{a_0} \right).
\]

The slope of the straight line represents \( \tau_0 \) which has a value of 
2.55 ± 0.2 h. The intersection of the straight line with the 
abscissa defines \( a_0 \). We found for this critical viral dose 0.8 ± 0.1 PFU/PMN.

These data again confirm that a change in cellular behavior 
occur above the critical virus dose \( a_0 \) resulting in a disturbance 
of chemotactic but not chemokinetic response.

**DISCUSSION**

Our experiments have yielded results in five areas of investi-
gation, which are discussed below.

**Ergoden Hypothesis**

On the basis of our results we have demonstrated that the 
degree of orientation, \( P_{1,\text{mono}} \), of one moving protein (time 
average) equals that of a population of cells, \( P_{1,\text{pop}} \) (average of 
a cell population). This finding confirms the Ergoden hypoth-
thesis that was originally formulated by Boltzmann (14) for 
moving molecules of a homogeneous population. To be certain 
that one is dealing with a homogeneous cell population, it is 
still necessary to compare individual cells or small groups of 
cells with populations of cells. Additional information about 
the homogeneity of cell populations is in general available 
from analysis of the distribution functions of different experi-
mental data. For example, analysis of the angular distribution 
function, \( N(\phi) \), of control PMN exposed to the chemotactic 
gradient revealed a single gaussian curve without evidence for 
subpopulations (Fig. 5a). Therefore we conclude that granu-
locytes moving in a chemotactic gradient behave as an ergodic 
system. This is of practical importance: Statistical predictions 
regarding the directed cell movement can be made for an 
individual cell even when the data have been gathered from a 
population of cells.

Analysis of the angular distribution function, \( N(\phi) \), of the 
protein-treated PMN in Fig. 5b reveals four items of information:

**FIGURE 9 Virus dose-dependent changes of the characteristic time, \( \tau \).**
(a) \(N(q)\) discloses two types of distribution functions: one peaks around 0°, as we found for control granulocytes, whereas the other population is broadly distributed in the ±180° sector. This indicates that we are dealing with an inhomogeneous cell population. (b) The percentage of both cell populations can be calculated: about one-third of the virus-treated PMN are in the ordered state like control granulocytes, whereas the remainder of the virus-treated PMN shows virus-induced disorder for this special experimental condition. (c) If we analyze \(P_{\text{pop}}\) of the first population (ordered state) of Fig. 5 b, we obtain a \(P_i\) degree of orientation of ~0.8, whereas the degree of orientation, \(P_{\text{pop}}\) of the other population is zero. The average degree of orientation of the whole cell population in Fig. 5 b is \(P_i = 0.26\). This demonstrates that Eq. 14 does not hold for this experimental condition. Therefore we can not make any statistical prediction for a single cell if the data are gathered from the whole cell population. However, statistical predictions for the whole cell populations are permitted. (d) From the results of Fig. 5 b we must conclude that according to Eq. 14 the Ergoden hypothesis does not hold for this experimental condition. However, we know from our data of Figs. 8 and 9 that the degree of orientation of virus-treated PMN declines to zero with a characteristic time, \(\tau\) (\(\tau_0 \sim 2.5\) h). In this virus-induced final state of PMN, again, we are dealing with a homogeneous cell population, and we are allowed to apply the Ergoden hypothesis.

Thus, application of the above mentioned criteria to the analysis of different experimental conditions, e.g., viral dose or time of virus interaction with PMN, permits the identification of cell subpopulations including their percentages, and also the investigation and description of time- or dose-dependent functional changes of target cells.

Degree of Orientation

The definition of the degree of orientation of an individual granulocyte or of a population of cells is given by the mathematical system itself. McCutcheon (15) characterized the oriented cell movement also by the system itself: he compared the distance \(D\) parallel to the chemotactic gradient with the actual path length \(L\) of the moving granulocyte (Fig. 2). The McCutcheon index \(D/L\) is identical to the degree of orientation of our model. This agreement can be confirmed by the following analysis. The real path length \(L\) can be divided in small path lengths \(\Delta s\):

\[
\Delta s = v_c \cdot \Delta t.
\]  

The actual path length is then

\[
L = N_0 \cdot \Delta s.
\]  

The projection of the small path length \(\Delta s\) onto the direction of the chemotactic gradient \(\Delta s_p\) is

\[
D = N_0 \cdot \Delta s_p = \sum \Delta s \cdot \cos \phi_p.
\]  

This equation can be rewritten using Eq. 20:

\[
D = L \cdot \langle \cos \phi_p \rangle,
\]  

QED.

The degree of orientation obtained by analyzing a single granulocyte is 0.85; the McCutcheon index of the same cell is 0.84; the degree of orientation of the population of cells is 0.85. As expected, the McCutcheon index is in accordance with the degree of orientation. Nossal and Zigmond (16) introduced the chemotropism index. Their description is in accordance with our Eq. 16.

Another parameter for estimating chemotactic reactivity of granulocytes that is mainly used in the literature is the determination of the percentage of oriented cells in the chemotactic gradient. For example, Zigmond (2) counted all granulocytes oriented in a ±90° sector around the chemotactic gradient divided by the total number of cells. This seems representative because 60% of the PMN are oriented within ±30° if a system is chosen with 0.85 degree of orientation. In this parameter some oriented cells are excluded, resulting in a relatively high percentage of oriented PMN. In contrast we counted all cells in a ±180° sector. Zigmond (2) obtained for one set of experimental conditions a percentage of oriented cells of 97%. We analyzed her results in retrospect with our method and arrived at a degree of orientation of 0.85. Furthermore, this comparison shows that the percentage of oriented cells is not equal to the McCutcheon index or the degree of orientation. This is mainly because the definition of the percentage of oriented PMN used by Zigmond and others (2, 8, 13, 17, 18) is not defined by the system itself.

Determination of Local Chemotactic Gradient

The degree of orientation is derived from the angular distribution function \(N(q)\). However, \(N(q)\) yields much more information about the system. Therefore, it is possible to examine whether the direction of the local chemotactic gradient is in accordance with the direction of the macroscopically applied chemotactic gradient (mean gradient). The directions of the local and the mean gradient are equal if the angular distribution function \(N(q)\) is symmetrical around zero (Fig. 4). This situation was assumed in Eqs. 8 and 9, and leads to zero for the average of \(\sin q\). However, under experimental conditions there may be small deviations in the local direction of the chemotactic gradient in comparison with the mean direction. In this case the average of \(\sin q\) is unequal to zero. This indicates that the evaluation of an experiment should only be performed if \(\langle \sin q \rangle\) is zero or less than the statistical error that is in the range of 0.05. To check the stability of the local chemotactic gradient, we determined \(\langle \sin q \rangle\) and \(\langle \cos q \rangle\) as a function of time (Fig. 6). As already mentioned by Zigmond (2), the chemotactic gradient is not established before 10 min after starting the experiment (dashed line in Fig. 6). As expected, \(\langle \sin q \rangle\) and \(\langle \cos q \rangle\) scattered around zero. The local chemotactic gradient is stable during the next 20 min since \(\langle \sin q \rangle\) was within the range of statistical error. However, at 45 min in this experiment \(\langle \sin q \rangle\) showed greater variations indicating local changes of the chemotactic gradient. The time dependence of \(\langle \sin q \rangle\) exhibits a trend that indicates a developing disturbance of experimental conditions, although the degree of orientation remains stable or even increases.

Directed Movement

Up to now we discussed (a) the degree of orientation, \(P_i\), which represents basically only a static information, and (b) the track velocity, \(V_c\), which describes the speed of nondirected movement. According to Eq. 11, both values are connected and their product, \(V_c\), defines the directed movement (chemotaxis). Insertion of the different experimental values of \(V_c\) and \(P_i\) into Eq. 11 yields the following results of \(V_c\).


\[ P_1 \times V_1 = V_t, \]
\[ 0.80 \times 30 \ \mu\text{m/min} = 24.0 \ \mu\text{m/min} \ 37^\circ\text{C}, \]
\[ 0.40 \times 5 \ \mu\text{m/min} = 2.0 \ \mu\text{m/min} \ 22^\circ\text{C}, \]
\[ 0.26 \times 30 \ \mu\text{m/min} = 7.8 \ \mu\text{m/min} \ \text{ECHO 9 A. B. virus} \]

(result from Fig. 5b).

These data demonstrate that \( V_1 \) and \( P_1 \) exhibit different sensitivities with respect to the virus and temperature. From these findings we conclude that the data processing for \( V_1 \) is at least partly different from those of \( P_1 \). This means that a change in the chemotactic response of PMN described by \( V_1 \) can be due either to changes of the track velocity \( V_1 \) (as in the case of \( 22^\circ\text{C} \)) or to changes of \( P_1 \) (as in the case of \( 22^\circ\text{C} \) or ECHO 9 A. B. virus). That means that for better control of experimental conditions and to avoid misinterpretations of in vitro results, not only the degree of orientation, \( P_1 \) but also \( V_1 \) and/or \( V_t \) should be determined.

**Virus and Directed Movement**

The chemotactic response of moving granulocytes is disturbed by ECHO 9 A. B. virus. We found that the track velocity of virus-treated PMN is not lowered; however, the degree of orientation declined. Debets-Ossenkopp et al. (19) used a different in vitro assay (under-agarose technique) for studying the interaction of influenza A virus with human PMN. They also found a decreased chemotactic response of virus-treated granulocytes. It is possible to interpret their results with our mathematical model: Under the likely assumption that \( V_0 \) is constant in their experiments, the degree of orientation is decreased by a factor of 0.42 indicating, too, a shift from chemotactic to chemokinetic cellular response.

We demonstrated that the degree of orientation declines with increasing virus dose under otherwise constant experimental conditions. This result underlines the pathogenetic importance of the virus-induced change in cell behavior. A further observation confirms the pathogenetic role of virus: At a given dose the degree of orientation declines with the increase in duration of virus-granulocyte interaction. The degree of orientation decays with time exponentially. It was possible to define a critical virus/granulocyte ratio of \( 0.8 \pm 0.1 \text{ pfu/PMN} \), indicating that there are two different states of virus-granulocyte interaction. At a virus dose below this critical value there is no disturbance of chemotactic response. But we do not know which other cellular events are influenced. Above this critical dose there is a virus dose-dependent decrease of the degree of orientation where the mean velocity is not affected. So far, the virus-induced disturbance of chemotactic response is described by Eqs. 11, 17, and 18 leading to a better understanding of virus-PMN interaction.

All our experimental data of the granulocyte-virus interaction with regard to the disturbed chemotactic cellular response are summarized in Eq. 18. This equation describes the dependence of the characteristic time, \( \tau \), on the viral dose, \( a \). Rewriting Eq. 18, we get an equation that has the same structure as the Weber-Fechner law (27, 28):

\[
\ln a - \ln a_0 = \frac{\tau_0^2}{\tau}. \tag{23}
\]

The Weber-Fechner law connects the stimulus difference with the perceptive intensities. In our case, \( \ln a_0 \) defines the threshold value of the virus stimulus for the PMN. Consequently, \( \ln a \) describes the virus stimulus for the granulocyte. The difference between any virus stimulus and the threshold value of virus stimulus is yielded by the square of the ratio \( \tau_0/\tau \). \( \tau \) describes the characteristic virus-induced decay time of the degree of orientation for a fixed viral dose, \( a \). Analogous with the Weber-Fechner law, \((1/\tau)^2\) can be used as the perceptive intensity characterizing the virus-PMN interaction. However, \( \tau_0 \) is not the virus-induced characteristic decay time of the threshold viral dose, \( a_0 \), because in this case the characteristic time is infinite. At this point, there is a deviation from the Weber-Fechner law. However, the characteristic time, \( \tau_0 \), occurs at a fixed viral dose that is \( e \)-times the viral threshold dose, \( a_0 \) \((a_0 \times 2.718 \ldots \). In the Weber-Fechner law the perceptive intensity is normally derived from subjective information. In contrast, the virus perception of PMN is derived from objective experimental data obtained from the virus dose- and time-dependent changes of the degree of orientation. So far, the virus stimulus, \( \ln a \), and the perceptive intensity, \((1/\tau)^2\), are based on objective measurements.

Aside from the general interest of our experimental findings about the interaction of viruses with moving cells, some practical clinical aspects have to be discussed: Several studies have indicated that there is a transient depression of leukocyte locomotion during the course of a viral infection (20). Several in vitro investigations with a variety of viruses confirm these in vivo results (7, 19, 20). Experience in the daily work in human and experimental pathology tells us that viral infections are histologically characterized by a nonpurulent (i.e., weak or absent granulocytic) inflammation (21, 22). Many viral infections, e.g., influenza virus, predispose to bacterial superinfections (19, 23). It has been suggested that the increased susceptibility to bacteria is due to the effect of viruses on the immune system and on phagocytic cells (24–26). The demonstrated disturbances of granulocyte function in our investigations support this theory. The selective inhibition of chemotactic activity of virus-treated PMN provides a basis for the understanding of the morphological and clinical findings in viral disease.

**Conclusion**

By means of our experimental assay and using our statistical model, the McCutcheon index and the chemotropism index can be placed in a better perspective. Besides the analysis of static values of the experimental assay of moving PMN, more information about dynamic properties of the in vitro system is available, leading to a better description of experimental conditions. Consequently, application of this model to clinical investigations e.g., ECHO 9 A. B. virus interaction with human granulocytes results in a more vigorous description and interpretation of experimental findings.

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