Eur Biophys J (1988) 16: 313–319

European Biophysics Journal

Galvanotaxis of human granulocytes

Dose-response curve

B. Rapp [1], A. de Boisfleury-Chevance [2], and H. Gruler [1]

[1] Abteilung für Biophysik, Universität Ulm, Oberer Eselsberg, D-7900 Ulm, Federal Republic of Germany
[2] Centre d'Etudes sur l'Ecologie des Cellules du Sang, Département d'Hématologie, Hôpital de la Salpétrière, 47 Boulevard de l'Hôpital, F-75013 Paris, France

Received February 4, 1988/Accepted in revised form September 2, 1988

Abstract. The galvanotactic response of human granulocytes was investigated theoretically and experimentally. The basic results are: (*i*) The granulocytes move towards the anode. (*ii*) The directed movement has been quantified by two different polar order parameters – the McCutcheon index and the average of $\cos\phi$. (*iii*) The polar order parameters are a function of the applied electric field (=dose-response curve). (*iv*) The inverse of the galvanotactic constant of migrating cells (analogous to the Michaelis-Menten constant) has a value of -0.2 ± 0.03 V/mm. (*v*) The galvanotactic response of granulocytes is a non-cooperative process with a cooperativity coefficient of 1 ± 0.2. (*vi*) The galvanotactic constant is a function of pH. (*vii*) The protein essential for the galvanotactic response is very likely a G-protein.

Key words: Galvanotaxis, granulocytes, dose-response curve

I. Introduction

Polymorphonuclear leukocytes (=granulocytes) are attracted by sites of inflammation to combat invading microorganisms. There must exist some mechanism to account for the fact that these cells move toward, and remain in, the vicinity of the sites of infection. The direction-determining mechanism(s) involved must be a function of some information transmitted to the leukocytes by the infected cells.

The signal may be chemical in nature; the resulting directed locomotion is then the chemotaxis. The peptides, in which the initial part of the molecule is a formylmethionyl moiety form a class of molecules which allow the granulocytes to distinguish between prokaryote and eukaryote cells. This is because the proteins of the prokaryote cells start with formylmethionyl and those of the eukaryote cells with any amino acid (Lehninger 1975; Snyderman 1983).

The signal may also be electrical in nature; the resulting directed locomotion is then galvanotaxis. For instance, when a cell is lysed, ions inside and outside the cell diffuse to reestablish a concentration equilibrium. This diffusion process is an inverse function of the size of the ions. The different diffusion constants of the ions involved result in the separation of small and large ions, and a diffusion potential is created (Atkins 1986). The diffusion potential can be demonstrated in the following way: A non-toxic salt crystal is placed in a suspension containing, for example, a paramecium which exhibits galvanotaxis towards the cathode (Dryl 1963). When the crystal dissolves, the small cations and the large anions diffuse away with different diffusion constants. In the resulting diffusion potential the crystal acts as cathode, and the paramecium is attracted by the crystal. Another, even simpler, experiment is the following one: To the suspension containing the paramecium a drop of a solution having a pH higher than that of the suspension is added. The drop repels the cells since owing to the fast diffusion of the hydrogen ions, the drop acts as an anode. However, if the added drop has a pH lower than that of the medium, the parameciums are attracted since the drop acts like a cathode (Dryl 1963; Gooday 1981).

In intact cells the plasma membrane separates solutions having different ion concentrations, thus maintaining a potential difference. When the plasma membrane is lysed the electric field is no longer localized at the membrane but rather spreads over a relatively large area. Barker et al. (1982) measured the electric field in the vicinity of wounds and found about 0.2 V/mm. To our knowledge the diffusion potential created by a single cell has not been measured as yet. The response of leukocytes to damaged cells may involve such electric fields. Fukushima et al. (1953) showed that the granulocytes move towards the anode at high pH and towards the cathode at low pH.

The topic of this article is to investigate the galvanotaxis of granulocytes. The method is not restricted

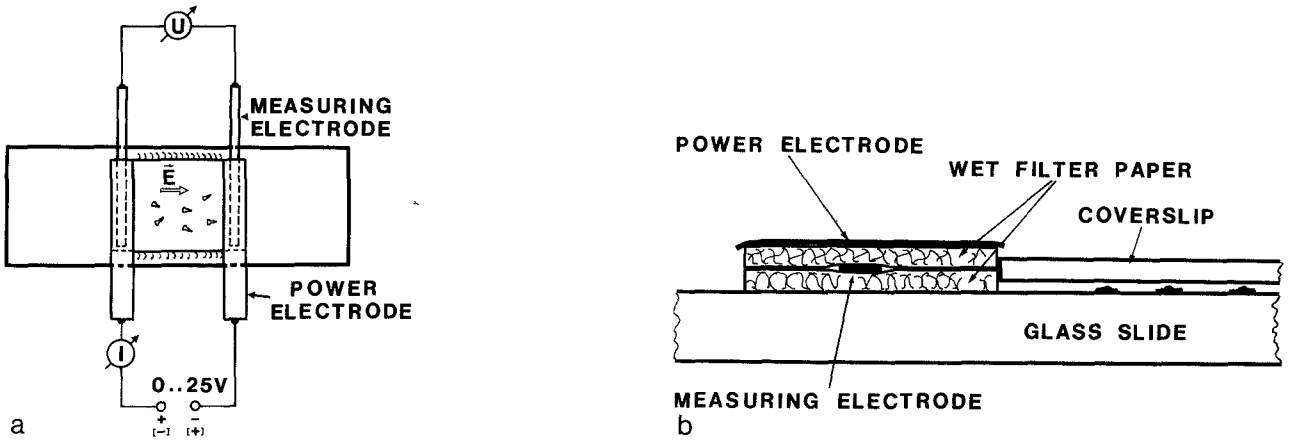

Fig. 1. **a** Top view to the chemotaxis chamber and the electric wiring. **b** Schematic drawing of the electrodes (cross section)

to granulocytes; the galvanotaxis chamber as well as the theoretical considerations can also be applied to other cell types in order to determine their galvanotactic dose-response curves.

II. Material and methods

1. Cell preparation

Human blood from a healthy donor was taken into heparin and sedimented by gravity for approximately 2 h. The buffy coat was suspended in 2.5 ml of autologous plasma and deposited on top of a 2.5 ml lymphoprep (Nyegaard, Oslo). The tube was centrifuged at 400 *g* for 30 min. The granulocytes were found enriched in a bottom layer. This layer was washed 3 times in the plasma of the same donor (first wash: 200 *g* for 10 min, second and third wash: 100 *g* for 10 min).

2. Galvanotaxis chamber

An electric field is created in the galvanotaxis chamber by sending an electric current through the aqueous solution (medium). There are two interfering factors. First, a voltage drop at the power electrodes, which makes it impossible to measure the electric field even when the chamber has a well-defined geometry. Second, products of electrolysis generated at the power electrodes diffuse through the medium and affect the movement of the cells. Thus the pH at the power electrode may alter dramatically under current flow. These problems can be eliminated by using an apparatus with four electrodes, two power electrodes and two measuring electrodes.

A drop of the cell suspension is transferred to a glass slide and then covered with a coverslip (20 mm · 20 mm). The drop must be very small so that the suspension driven by capillarity makes only a thin (10 – 20 μm) film between slide and coverslip. The coverslip is attached with paraffin to the glass slide on two opposing sides (Fig. 1 a) and two assemblies consisting of filter paper (10 mm · 20 mm), measuring electrode (aluminium, 50 mm · 2 mm · 0.1 mm), filter paper (10 mm · 20 mm), power electrode (aluminium 50 mm · 12 mm · 0.1 mm) are attached to the open sides of the slide-and-coverslip preparation (Fig. 1 b). The damp bottom layer of filter paper must be in direct contact with the cell suspension. Excess liquid should be avoided to prevent movement of the coverslip and of the cell. The whole block with the electrodes is sealed with paraffin.

The power electrodes are connected with an electric power supply (0 ... 25 V) with alterable polarity. The electric current is less than 1 mA for a field of 1 V/mm. Changes in current indicate changes in experimental conditions (e.g. thickness changes of the liquid film, drying-out of the electrodes). The measuring electrodes are connected to a voltage meter having a high resistivity ($\geq$ 10 MΩ). The electric field strength in the thin liquid film is the measured voltage divided by the side length of the coverslip. The resistance of the electrode assembly is low enough to be neglected. The disadvantage of this apparatus is that products of electrolysis are not prevented from entering the chamber. Therefore, the measuring time must be kept short ($\approx$10 min). Longer measuring times can be achieved by periodically changing the polarity.

3. Collection and analysis of the data

The galvanotaxis chamber was placed on the heating stage of a phase contrast microscope. All the experiments were performed at 37 °C and the cell movement was recorded in real time on a video tape.

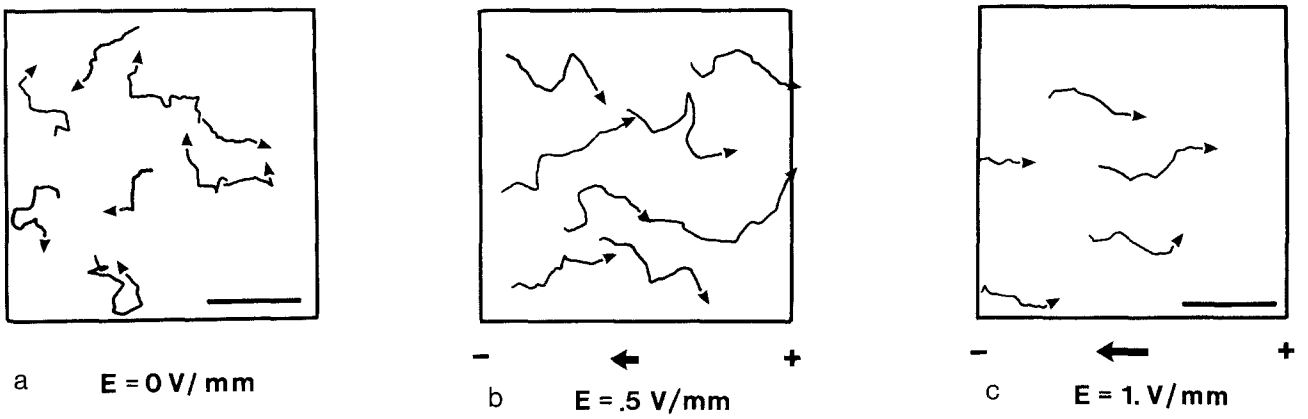

Fig. 2a–c. Trajectories of migrating granulocytes exposed to different electric field strengths. The *bar* corresponds to 50 μm. **a** 0 V/mm exposure time 435 s; **b** 0.5 V/mm exposure time 225 s; **c** 1 V/mm exposure time 195 s

The directed movement can be quantified if the center of gravity or the orientation of the migrating cells (Zigmond 1977) are known. The orientation and the center of gravity approximated by the center of the cell area were determined from the contour line of the cell by computerized image analysis (IBAS II, Kontron, Munich). The interval between consecutive images was 15 sec. The average from two or more frames was taken when the shape of the cell was appreciably changing with time.

The migrating cells drift parallel to the electric field they are exposed to so that the mean displacement, $\langle x \rangle$, is the average drift velocity, $\langle v_{II} \rangle$, quantifying the directed movement, multiplied by the time t.

$$\langle x \rangle = \langle v_{II} \rangle \cdot t. \tag{1}$$

As previously shown (Bültmann and Gruler 1983; Lauffenberger et al. 1983; Gruler 1984), the mean drift velocity, $\langle v_{II} \rangle$, is the product of the mean track velocity, $\langle v_C \rangle$, and the average of $\cos\phi$ where ϕ is the angle between the direction of migration and the electric field,

$$\langle v_{II} \rangle = \langle v_C \rangle \cdot \langle \cos\phi \rangle. \tag{2}$$

The directed movement can be characterized by two parameters (Gruler and Bültmann 1984a and b): (*i*) The chemotropism index $\langle v_{II} \rangle / \langle v_C \rangle$, which is identical with the McCutcheon index (ratio between travel parallel to the electric field and the actual distance travelled) and (*ii*) the average of $\cos\phi$

$$\langle \cos\phi \rangle = \frac{1}{(2\pi)} \int_0^{2\pi} f(\phi) \cdot \cos\phi \cdot d\phi \tag{3}$$

with the angle distribution function $f(\phi)$.

A general term – the polar order parameter ($= \langle P_1 \rangle$) – can be introduced as long as Eq. (2) is valid. This polar order parameter can be determined either by the McCutcheon (chemotropism) index, or the average of $\cos\phi$. In this publication, the McCutcheon index and the average of $\cos\phi$ are determined separately from the video tapes.

The polar order parameter equals 0 for random movement and 1 if the cells move in a straight line parallel to the electric field. The polar order parameter is positive when the cells migrate towards the cathode and negative when the cells migrate towards the anode. Values between 0 and -1 therefore indicate the extent to which cells move towards the anode.

III. Results and discussion

The random movement of granulocytes as shown in Fig. 2a was obtained when no voltage was applied. The mean displacements in the x and y direction divided by the measuring time t (see also (1)) are zero ($\langle x \rangle / t = \langle y \rangle / t = 0 \pm 0.8$ μm/min, 9 cells, $t = 30$ min, $\langle v_C \rangle = 15$ μm/min) and the average of $\cos\phi$ is zero ($\langle \cos\phi \rangle = 0 \pm 0.07$) as expected for an isotropic cellular environment. The random movement of the cells became directed when an electric field was applied. The paths appeared snake-like and led to the anode (Fig. 2b). The McCutcheon index and $\langle \cos\phi \rangle$became unequal to zero and increased monotonically with increasing electric field strength (Fig. 3a and b). These two independent measured parameters are nearly identical. This was expected since we showed previously in a necrotactic experiment (Gruler 1984) that the McCutcheon index equals $\langle \cos\phi \rangle$. We use the term polar order parameter for describing the directed movement when it is not necessary to show how the parameter was determined. To our knowledge this is the first published dose-response curve of granulocytes exposed to an electric field. Our measurements are in accordance with that of Fukushima et al. (1953). We determined from their published data the McCut-

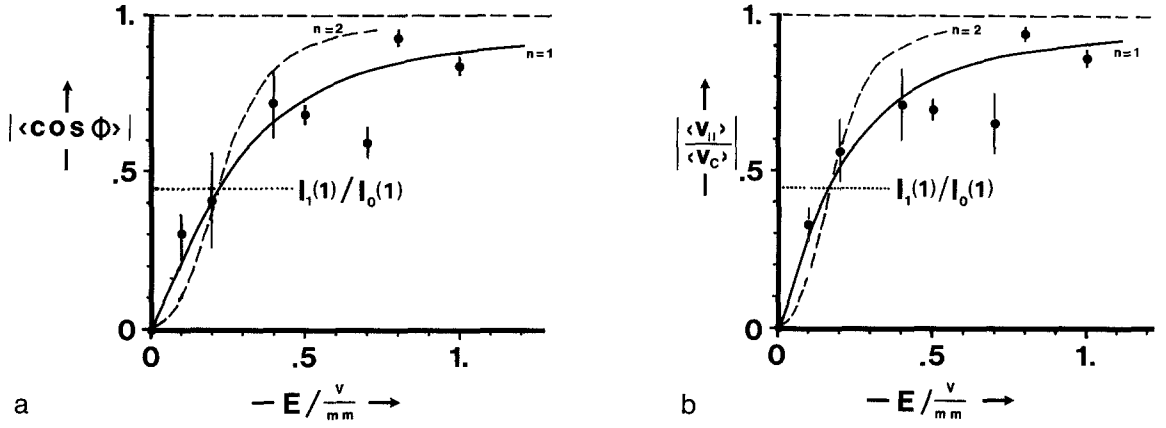

Fig. 3a and b. Galvanotactic dose-response curves of migrating granulocytes. The dose is the applied electric field strength E and the cellular response is in (**a**) the average of the cell orientation ($=\langle\cos\phi\rangle$) and in (**b**) the McCutcheon index. The error bars are the standard error of the mean. The typical number of observed cells per data point is between 10 and 15. The drawn and the dashed line are theoretical predictions (4) and (5) for a non-cooperative process ($n=1$, *drawn line*) and for a cooperative process ($n=2$, *dashed line*)

cheon index to be -0.67 ± 0.05 at a field strength of ≈ 0.4 V/mm. (The electric field used by Fukushima et al. (1953) is estimated from the conductivity of the medium, the applied current and the sample geometry.) This value fits quite well into Fig. 3a and b.

We now compare the measured dose response curve with mathematical expressions derived previously (Gruler and Nuccitelli 1986; Gruler 1988a and b).

$$\langle P_1\rangle = \langle\cos\phi\rangle = \frac{\langle v_{II}\rangle}{\langle v_C\rangle} = \frac{I_1(a_1)}{I_0(a_1)} \tag{4}$$

$$a_1 = (K_G \cdot E)^n. \tag{5}$$

I_1 and I_0 are hyperbolic Bessel functions (Abramowitz and Stegun 1964). Their arguments depended on the electric field strength, E, times the galvanotactic constant, K_G. The sensitivity of the cell to the electric field strength and the cooperativity of the cellular response are described by the galvanotactic constant, K_G, and the cooperativity coefficient, n, respectively.

The galvanotactic constant and the cooperativity coefficient can be obtained by fitting the experimental data to the theory. Galvanotactic response curves for a cooperative (thin line, $n=2$) and for a non-cooperative (thick line, $n=1$) process are shown in Fig. 3a and b. The hyperbole-like curve for the non-cooperative process approximates the experimental data much better than the sigmoidal curve for the cooperative process. The galvanotactic response is obviously a non-cooperative process with a galvanotactic constant of $(-0.22\ \text{V/mm})^{-1}$ for the $\langle\cos\phi\rangle$ data and $(-0.17\ \text{V/mm})^{-1}$ for the chemotropism index data. The galvanotactic constant hence is $(-0.2 \pm 0.03\ \text{V/mm})^{-1}$.

There are similarities between the chemotactic and the galvanotactic responses. Chemotaxis is described by (4) and another equation similar to (5) (Gruler 1988a and b). Brokaw (1958) was the first to show how to compare the two phenomena by using $E/(\text{grad ln } c) = \text{constant}$, where c is the concentration of the chemoattractant molecule and he proved the validity of the equation for bracken spermatozoids of fern. Our analysis of Brokaw's galvanotactic dose-response curve (Brokaw 1959) gives a cooperativity coefficient of one (Gruler 1988a and b). The similarity of the response curves holds also for granulocytes. To show this we analysed Zigmond's chemotactic data (1977). As in galvanotaxis, we found a non-cooperative response curve when the mean concentration of the chemoattractant was kept constant (Gruler 1988a and b). For different mean concentrations we found that the chemotactic constant, K_{CT}, is a function of the mean concentration of the chemoattractant molecule, c_0, ($K_{CT} = A(c_0/K)(1 + c_0/K)^{-2}$, where A is a constant and K is the equilibrium binding constant of the chemotactic molecule to the receptor). This concentration dependent chemotactic constant can be explained if one assumes that the granulocyte measures at one time the concentration of the chemotactic molecule at two places in the plasma membrane and compares the results (Gruler 1988a and b). Our previous conclusion that the chemotactic response is cooperative (Gruler and Nuccitelli 1986) is not correct.

Directed movement is only possible when the cell measures the electric field strength and guides its movement according to the measured value. We don't know the nature of the cell's own standard E_0 for electric field strength but we know its value measured in V/mm. The field strength of the cellular standard E_0 is obtained for $a_1 (= E_0 \cdot K_G) = 1$. The inverse of the galvanotactic constant K_G can be regarded as the cellular standard for electric fields. This idea is related to one with which everyone is familiar: The dimensionless concentration in the Michaelis-Menten equation is the substrate concentration divided by the Michaelis-Menten constant. From the viewpoint of

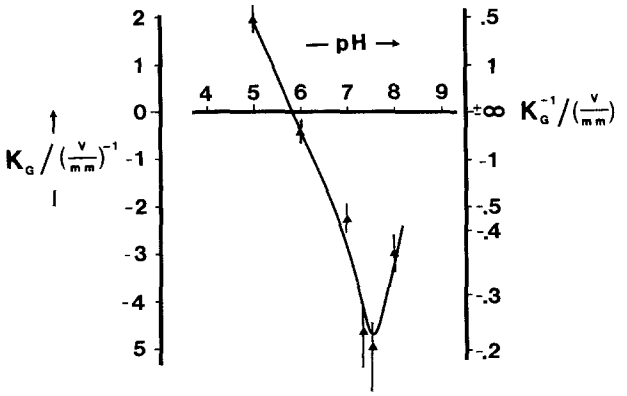

Fig. 4. Galvanotactic constant as a function of pH. Experimental data of Fukushima et al. (1953) were used

the enzyme, the substrate concentration is measured in units of the Michaelis-Menten constant. As expected, different cell types have different galvanotactic constants: -0.2 ± 0.03 V/mm for human granulocytes; $+0.3 \pm 0.015$ V/mm for fibroblasts (Gruler and Nuccitelli 1986); 0.024 V/mm for bracken spermatozoids (Gruler 1988 a and b).

A change in the chemical environment leads to an altered value of the chemotactic constant. This fact is very well-known in enzyme kinetics. For example, a change in ion concentrations in the vicinity of the enzyme leads in general to an altered Michaelis-Menten constant. The same phenomenon may happen in directed cell movement. One expects that the galvanotactic constant changes its value in different chemical environments. Let us assume the cellular environment is constant except for the pH value[1], then the polar order parameter is also a function of pH,

$$\langle P_1(E; \mathrm{pH}) \rangle = \frac{I_1(a_1(E; \mathrm{pH}))}{I_0(a_1(E; \mathrm{pH}))} \tag{6}$$

$$\text{with } a_1(E; \mathrm{pH}) = (E \cdot K_G(\mathrm{pH}))^n. \tag{7}$$

Fukushima et al. (1953) found that the galvanotactic response is indeed a function of pH. The response is optimum at pH 7.5. We determined from their data the McCutcheon index to be $+0.36$, -0.08, -0.41, -0.67, -0.70, -0.50 (estimated error ± 0.05) for pH values 5.0, 6.0, 7.0, 7.3, 7.5, 8.0, respectively. Under the assumption that the cooperativity is independent of the pH one gets the galvanotactic constant as a function of pH. The results are shown in Fig. 4. The galvanotactic constant is minimum at pH 7.5 with a value of $(-0.2 \text{ V/mm})^{-1}$ as in our experiment. The decay of K_G is very dramatic: $(-0.42 \text{ V/mm})^{-1}$ for pH 7 and $(-2.2 \text{ V/mm})^{-1}$ for pH 6. Surprisingly, at pH 5 the galvanotactic constant is positive $(+0.5 \text{ V/mm})^{-1}$. Obviously, the galvanotactic process has an isolectric point at pH 5.8. At pH > 5.8, the cells migrate towards the anode and at pH < 5.8, the granulocytes move towards the cathode.

The isoelectric point of enzymes can be used to characterize enzymes. The action spectrum of galvanotaxis as a function of pH can be interpreted by the isoelectric point of proteins essential in galvanotaxis. These proteins will be restricted to only a few having the right isoelectric point. The G-proteins are a family of GTP binding regulatory proteins which couple receptor – mediated stimulation to different effector enzyme systems and ion channels involved in the response of a wide variety of cells (Stryer and Bourne 1986). Becker et al. (1987) determined the isoelectric point of the G-protein of granulocytes to be pH 5.7. The accordance between the isoelectric point of the G-protein with the isoelectric point in the action spectrum is a strong hint that the membrane-bound G-protein is essential in galvanotaxis. It is likely that the molecular machine used in galvanotaxis is also the molecular machine used in chemotaxis minus the receptor for the chemoattractant molecules.

Fromherz (1988) developed a model for the cellular decision which fits quite well the galvanotactic and chemotactic response data. He connected the cable equation (Hille 1984) which is derived, for example, for nerve excitation, with mobile ion channels. Here we have to assume that the charged G-protein forms a functional unit with the ion channels. In galvanotaxis, the cable equation has to be considered since we are dealing with an electrical phenomenon. The granulocyte plasma membrane is a liquid crystal and hence functional units can diffuse in it (fluid mosaic model). The density of the G-protein and the electric field pattern are coupled since G-proteins having a net charge are driven by the electric field component parallel to the membrane surface. This electric field generated by the distribution of the ion channels is described by coupled non-linear differential equations.

The interesting fact is that the system can either be in a stable or in an unstable state depending on parameters like those of the ion pumps. One has here the same situation as in oscillating chemical reactions (Haken 1983). The leading front formation as a capping phenomenon can be understood as the uphill diffusion of the functional units. The system can have a positive feedback so that the functional units are concentrated within a small region. The localized ion current was measured for many large cells but measurements on small cells like granulocytes are hampered by experimental difficulties (Nuccitelli 1983). An important observation is that a localized ion current precedes the formation of the leading front in amoebae.

In the Fromherz model (1988) functional units will form a patch where the initial concentration is the

[1] The results are shown for different proton concentrations but the effect considered is not restricted to the protons. It holds for any molecule or ion

largest. Initially, the charged G-proteins show an electrophoretic drift within the membrane, driven by the external electric field, towards the anode or the cathode depending on the net charge of the G-protein (functional unit). The variation in the density of the functional units is very small since the driving electric field is very small. Then when the instability has taken place the concentration of functional units is very high and will form a patch which points towards the cathode or anode depending on the net charge of the G-protein.

The Fromherz model describes only the important initial step in galvanotaxis. The next step in the signal/transduction mechanism has to be considered as, for example, the role of Ca^{++} as second messenger.

IV. Concluding remarks

We showed that the galvanotactic response of human granulocytes can be considered in a way similar to that used in enzyme kinetics. The dose response curve of granulocytes exposed to an electric field can be described by two parameters, the galvanotactic constant and the cooperativity coefficient. The galvanotactic response of granulocytes is obviously a non-cooperative process. The galvanotactic constant of granulocytes is $(-0.2 \pm 0.03\ \mathrm{V/mm})^{-1}$. It is likely that the protein essential for the galvanotactic response is a membrane-bound G-protein.

The practical importance of this study lies in the detailed analysis of the experimental conditions. It provides an objective description of directed cell movement which may be useful in the definition of granulocyte dysfunction. For example granulocytes exposed to viruses can lose their ability for directed movement as Bültmann and Gruler (1983) have shown in a Zigmond chamber (chemotaxis). The virus-induced order-disorder transition can also be detected in galvanotaxis as we have found in preliminary experiments. Here we have to say that galvanotaxis is much faster and easier to measure than chemotaxis. The whole dose response curve can be obtained with a few galvanotactic chambers. Furthermore, the advantage of the technique employed is that it is not restricted to granulocytes. It is likely to be useful in similar investigations on the other cell types.

Acknowledgement. This work was supported by a NATO travel grant and by "Fond der chemischen Industrie".

Appendix

$\langle P_1 \rangle$ as a power series of electric field strength

The theoretical expression for the polar order parameter as a function of the applied electric field strength looks complicated for those not familiar with mathematics. A simple expression can be derived if the power expansions of the Bessel functions are used (Abramowitz and Stegun 1964).

$$\begin{aligned}\langle P_1(E)\rangle &= I_1(b)/I_0(b) = 3.75 \cdot b \cdot \{0.5 - 0.87890551 \cdot b^2 \\ &\quad + 2.0599178279 \cdot b^4 - 4.9786521469 \cdot b^6 \\ &\quad + 0.1209225531 \cdot b^8 - 0.2939512328 \cdot b^{10} \\ &\quad + 0.7146763929 \cdot b^{12} \pm \text{higher order terms in b}\}\end{aligned} \tag{A 1}$$

$$\text{with } b = (K_G \cdot E)^n / 3.75. \tag{A 2}$$

This approximation holds for $b \leq 1.8$.

Another analytical expression which holds for large electric field strengths ($b \geq 3.5$) is often very useful

$$\begin{aligned}\langle P_1(E)\rangle &= 1 - 0.1332678 \cdot b^{-1} - 0.0102841561 \cdot b^{-2} \\ &\quad + 0.0091506274 \cdot b^{-3} - 0.0495980164 \cdot b^{-4} \\ &\quad + 0.1134248555 \cdot b^{-5} - 0.1487365524 \cdot b^{-6} \\ &\quad + 0.0982901940 \cdot b^{-7} - 0.0255634910 \cdot b^{-8} \\ &\quad \pm \text{higher order terms in } b.\end{aligned} \tag{A 3}$$

A computer program (Turbo-Pascal) for calculating $\langle P_1(E)\rangle$ ($0 \leq |E| < +\infty$, and K_G and n are parameters) is available from the authors.

Slope of $\langle P_1(E)\rangle$

The slope of the polar order parameter, $\langle P_1(E)\rangle$, as a function of the electric field strength can be used to estimate the cooperativity coefficient, n

$$\frac{d\langle P_1(E)\rangle}{dE} = \frac{d\langle P_1(a_1)\rangle}{da_1} \cdot \frac{da_1}{dE} \tag{A 4}$$

$$\text{with } da_1/dE = n \cdot K_G \cdot (K_G \cdot E)^{n-1} \tag{A 5}$$

and

$$\frac{d\langle P_1(a_1)\rangle}{da_1} = \frac{1}{I_0} \cdot \frac{dI_1}{da_1} - \frac{I_1}{I_0^2} \cdot \frac{dI_0}{da_1} \tag{A 6}$$

The following features of the Bessel function are used (Abramowitz and Stegun 1964)

$$\frac{dI_1}{da_1} = I_0 - \frac{I_1}{a_1} \tag{A 7}$$

$$\frac{dI_0}{da_1} = I_{-1} \tag{A 8}$$

$$I_1 - I_{-1} = 0. \tag{A 9}$$

(A 4) reads by using (3), (A 7), (A 8), and (A 9)

$$\frac{d\langle P_1(a_1)\rangle}{da_1} = 1 - \frac{\langle P_1(a_1)\rangle}{a_1} - \langle P_1(a_1)\rangle^2. \tag{A 10}$$

The easiest way to determine the cooperativity coefficient is to measure the slope at $a_1 = 1$

$$\left.\frac{d\langle P_1(a_1)\rangle}{da_1}\right|_{a_1=1} = 0.355\ldots \qquad \text{(A 11)}$$

or

$$\left.\frac{d\langle P_1(E)\rangle}{dE}\right|_{E=K_G^{-1}} = n\cdot K_G\cdot 0.355\ldots \qquad \text{(A 12)}$$

References

Abramowitz M, Stegun IA (1964) Handbook of mathematical functions with formulas, graphs, and mathematical tables. National Bureau of Standards Applied Mathematical Series 55, pp 355

Atkins PW (1986) Physical Chemistry. Oxford University Press, Oxford, p 260

Barker AT, Jaffe LF, Vanable JW (1982) The glabrous epidermis of cavies contains a powerful battery. Am J Physiol. 242:R358–R366

Becker EL, Kanaho Y, Kermode JC (1987) Nature and functioning of the pertussis toxin-sensitive G-protein of neutrophils. Biomed Pharmacol 41:289–297

Brokaw CJ (1958) Chemotaxis of bracken spermatozoids. Implications of electrochemical orientation. J Exp Biol 35:197–213

Brokaw CJ (1959) Random and oriented movements of Bracken spermatozoids. J Cell Comp Physiol 59:95–101

Bültmann BD, Gruler H (1983) Analysis of the directed and nondirected movement of human granulocytes: influence of temperature and echo 9 virus on N-formyl methionyl leucyl phenylalanine induced chemokinesis and chemotaxis. J Cell Biol 96:1708–1716

Dryl SA (1963) Contribution to mechanism of chemotactic response on *Paramecium caudatum*. Anim Behav 11:393–396

Fromherz P (1988) Selforganization of the fluid mosaic of charged channel proteins in membranes. Proc Natl Acad Sci USA 85:6353–6357

Fukushima K, Senda N, Innui H, Mirua H, Tamai Y, Murakami Y (1953) Studies on galvanotaxis of leukocytes. Med J Osaka Univ 4:195–208

Gooday GW (1981) Chemotaxis in eukaryontic microbes. In: Lackie JM, Wilkinson PC (eds) Biology of the chemotactic response. Society for Experimental Biology, Seminar Series, vol 12. Cambridge University Press, London, pp 115–138

Gruler H (1984) Cell movement analysis in necrotactic assay. Blood Cells 10:107–122

Gruler H (1988a) Cell movement and symmetry of the cellular environment. Z Naturforsch 43c, 754–764

Gruler H (1988b) Biophysics of leukocytes: neutrophil chemotaxis, characteristics and mechanisms. In: Hallet MB (ed) The cellular biochemistry and physiology of neutrophil. CRC Press, Boca Raton, Florida

Gruler H, Bültmann BD (1984 a) Analysis of cell movement, Blood Cells 10:61–77

Gruler H, Bültmann B (1984 b) Virus-induced order-disorder transition of moving human leukocytes. Nuovo Cimento (Suppl) 3D:152–173

Gruler H, Nuccitelli R (1986) New insights into galvanotaxis and other directed cell movements: an analysis of the translocation distribution function. In: Nuccitelli R (ed) Ionic currents in development. Alan R Liss, New York, pp 337–347

Haken H (1983) Synergetics. Nonequilibrum phase transitions and self-organization in physics, chemistry, and biology. Springer, Berlin Heidelberg New York

Hille B (1984) Ionic channels of excitable membranes. Sinauer, Sunderland, MA

Lauffenburger D, Rothman C, Zigmond SH (1983) Measurement of leukocyte motility and chemotaxis parameters with a linear under-agarose migration assay. J Immunol 131:940–947

Lehninger AL (1975) Biochemistry. Worth, New York

Malvista SE, de Boisfleury-Chevance A (1982) The cytokineplast: purified, stable and functional machinery from human blood polymorphonuclear leukocytes. J Cell Biol 95:960–973

Nuccitelli R (1983) Transcellular ion currents: Signals and effectors of cell polarity. Mod Cell Biol 2:451–481

Snyderman R (1983) Pharmacologic manipulation of leukocyte chemotaxis. Present knowledge and future trends. Am J Med 31:10–18

Stryer L, Bourne HRG (1986) G-proteins: a family of signal transducers. Annu Rev Cell Biol 2:391–419

Zigmond SH (1977) Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. J Cell Biol 75:606–616