TRAJECTORIES OF HUMAN GRANULOCYTES

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ABSTRACT The spatial trajectories of human granulocytes moving on glass in two dimensions under an isotropic medium may be characterized as realizations of a correlated walk. The present model is similar to an earlier one for slime-mold amebae. The correlation from step to step is essentially via the relative angle θ, represented for granulocytes as a random variable with a symmetric bimodal density. As the cells age, they appear to turn less and the degree of correlation increases. Even under chemotaxis the angle θ and the step length r appear to be statistically independent.

INTRODUCTION

In this paper we propose a correlated walk model (Fürth, 1920; Tchen, 1950; Klein, 1952; Patlak, 1953; Barber and Ninham, 1970; Nossal and Weiss, 1974*a*; Hall, 1977) as a characterization for the paths of human granulocytes in the plane. Our model is based on an experimental study in which granulocytes were placed on glass in an artificial medium and their positions recorded by time-lapse photomicrography. The experimental work was carried out by Dr. P. Noble and the techniques used have been described in detail in Noble and Peterson (1972), Peterson and Noble (1972), Boyarsky et al. (1976), and Hall (1977).

The trajectory of one of the cells whose paths we have analyzed to construct our model is shown in Fig. 1. The idealization of the path into a sequence of straight-line steps follows from the experimental rule that the cell has turned only if it deviates from an approximately straight path by more than half a cell diameter. In practice this means that "turns" of less than about $\pm 8°$ are ignored. We have described this segmental representation of cell-paths earlier in connection with the movement of slime-mold amebae (Hall, 1977, Section 3). The granulocytes stop for varying periods of time; they usually stop before turns and occasionally during straight-line movement. During continuous movement the velocities are not usually constant. However, the present work is concerned only with the spatial trajectories of the cells and the epoch in our stochastic model is the step number n.

If alternatively, the positions of the cell were recorded at equal intervals τ of time and these points were joined by straight lines, then one would again have a sequence of segments for which a stochastic model could be designed, the epoch now being time (in units of τ). Such models have been constructed (Gail and Boone, 1970; Nossal and Weiss, 1974*b*; Lovely and Dahlquist, 1975) but they do not characterize the actual cell-path in space. Although the task of introducing time into our spatial model may have to wait until stopping-times and velocities are better understood, the spatial trajectories for the slime-mold amebae and the human granulocytes which we have been able to study do themselves appear to be very interesting and quite distinct.

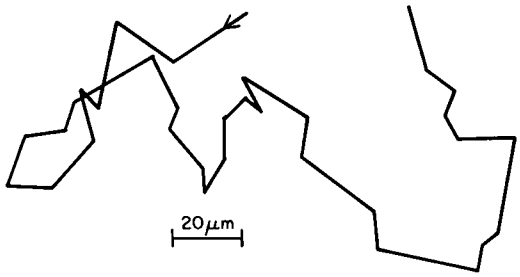

FIGURE 1 A granulocyte trajectory.

MEASUREMENT OF CELL MOVEMENT

For the present studies human granulocytes were obtained by a method first reported by Harris (1953). A drop of blood from a pricked finger was placed in the center of a microscope slide and incubated at 37°C in a moist atmosphere for 30 min. The clotted blood was carefully removed and the residual red blood cells were washed off quickly with Hanks' balanced salt solution. The remaining cells, approximately 95% granulocytes, were covered with a drop of medium made from 50% human serum (heat-activated) and 50% minimum essential medium (Eagle's salts), *N*-2-hydroxyethylpiperazize-*N'*-2-ethane sulfonic acid (HEPES)-buffered (pH 7.2). A well was formed with 1-mm-diameter glass capillary tubing or 1-mm-thick microscope slide fragments fixed to the microscope slide with a mixture of Histowax (Chesebrough-Ponds, Inc., Greenwich, Conn.) and Vaseline (1:1). The well was then filled with additional medium and a cover slip was cemented to the top of the well with the wax mixture.

Cells were filmed with a Wild M40 inverted microscope and a Wild time-lapse unit (Wild-Leitz Canada, Brossard, Quebec). One frame was exposed every 8 s with an exposure time of 1.5 s determined by an electromagnetic shutter that blocked the illuminating beam between exposures. A total optical magnification of 54 was obtained using low-power phase-contrast optics. The temperature of the preparation was maintained at 37°C with a Wild hot stage. The resulting 16-mm films were projected on to dimensionally stable paper with a Kodak analytical projector (L-M Photo, Van Nuys, Calif.) at a constant magnification. The center position of each cell (determined visually) was plotted for each frame and the centers were connected by a series of straight line segments. When a center point deviated from the extension of the straight line formed from a sequence of previous center points by more than half a cell diameter, a change in direction was considered to have occurred.

Cell trajectories thus formed were converted into digital data by determining the (x,y) coordinates of each point where a change in direction occurred. From these (x,y) coordinates, the mean step length μ_r, the angular change in direction between steps θ, the step number n, and the total displacement could be determined.

The filmed data used in this study were generously supplied by Dr. P.B. Noble.

THE CORRELATED WALK MODEL

The model we propose for granulocyte movement is of the same general type as the one we have used for slime-mold amebae (Hall, 1977). Suppose $\{r_i\}$ is a sequence of two vectors representing the steps in the trajectory of a moving cell. The position of the cell at the end of n steps is given by:

$$\mathbf{R}_n = \sum_{i=0}^{n-1} \mathbf{r}_i \qquad (1)$$

The step lengths, $r_i = |\mathbf{r}_i|$, are values of a nonnegative random variable with density $g(r)$, and the relative angles θ_i $(-\pi \leq \theta_i \leq \pi)$ between $\mathbf{r}_{i-1}$ and $\mathbf{r}_i$ $(i \geq 1)$ are values of a random variable with density $f(\theta)$ that is symmetrical (i.e., even) about $\theta = 0$. We assume that the relative angles are independent of each other, that the step lengths are independent of each other, and that the relative angles are independent of the step lengths. The initial direction ϕ_0, the angle between $\mathbf{r}_0$ and the x-axis, is assumed to have a uniform distribution on $[-\pi, \pi]$ and to be independent of the relative angles and step lengths.

We have analyzed data from the experimental studies of Dr. P. Noble, and Figs. 2 and 3 are histograms of θ values and step-lengths r made by 14 human granulocytes in a typical experiment. By applying the statistical tests described in Hall (1977) to this data, we are unable to falsify the main hypotheses of our model. For granulocyte movement under isotropic conditions the correlation between the steps appears to be essentially via the *relative angles* $\{\theta_i\}$; the relative angles and the step-lengths appear to be independent.

The θ-histogram (Fig. 2) is clearly bimodal and therefore qualitatively quite different from the corresponding unimodal bell-shaped histogram that we found for slime-mold cells (Hall, 1977). Although our definition of a step (distance between turns greater than about 8°) necessarily depletes the center region of the θ-histogram, the wide depression we find around $\theta = 0$ in Fig. 2 cannot be accounted for in this way. For slime-mold amebae we find $\overline{|\theta|} = 40°$, whereas for granulocytes we find $\overline{|\theta|} = 67°$; the granulocytes tend to turn through larger angles than the amebae. This difference between the two types of cell is also reflected in the value of the parameter $\lambda = \overline{\cos\theta}$, important in the theory of the correlated walk: for the amebae and the granulocytes we find $\lambda = 0.7$ and $\lambda = 0.3$, respectively.

The smooth curves superimposed on the histograms (Figs. 2 and 3) are graphs of probabil-

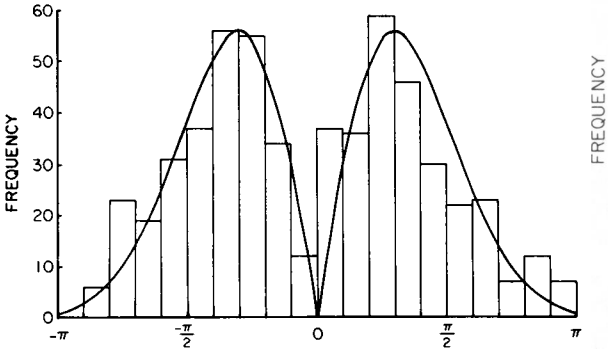

FIGURE 2

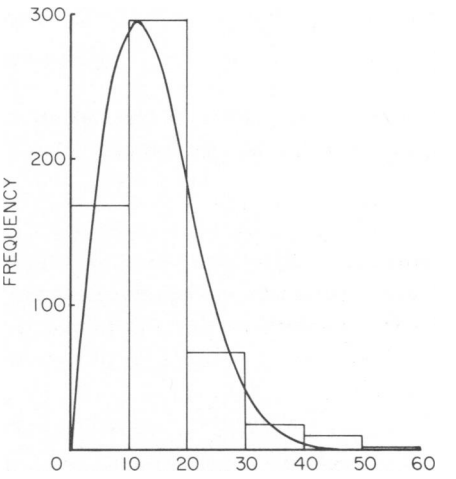

FIGURE 3

FIGURE 2 Histogram of θ, the relative angle, turned from step to step. The smooth curve is the model density $f(\theta)$ multiplied by the histogram area of 174 radians.

FIGURE 3 Histogram of r, the step length. The abscissa units are to be multiplied by the scale factor 1.06 to obtain lengths in micrometers. The smooth curve is the model density $g(r)$ multiplied by the histogram area of 4,044 μm.

ity densities (multiplied by the respective histogram areas), which can be used to represent the data within the framework of our model. These densities are convenient elementary functions whose first and second moments agree with those of the data to within 5% and whose shapes are similar. They represent a summary of the data we have studied in the reference frame of our correlated-walk model. The densities are given by:

$$f(\theta) = \begin{cases} (2\alpha^2)^{-1} \mid \theta \mid e^{-\theta^2/2\alpha^2}, & \mid \theta \mid \leq \pi, \quad \alpha = 0.94, \\ 0, & \mid \theta \mid > \pi; \end{cases} \tag{2}$$

$$g(r) = \begin{cases} \beta^{-2} r e^{-r^2/2\beta^2}, & 0 \leq r \leq 45\,\mu\text{m}, \quad \beta = 12\,\mu\text{m}, \\ 0, & r > 45\,\mu\text{m}. \end{cases} \tag{3}$$

The abscissa units in Fig. 3 are to be multiplied by the scaling factor 1.06 to obtain lengths in micrometers.

The joint density $p(r, \theta)$ in our model is given by

$$p(\theta, r) = f(\theta)g(r). \tag{4}$$

If we wish to view the sequence of steps of the correlated walk as an equivalent Markov chain, the appropriate conditional probability density P is given by:

$$P(\phi_i, r_i \mid \{\phi_k, r_k\}, k < i) = P(\phi_i, r_i \mid \phi_{i-1}, r_{i-1}) = f(\phi_i - \phi_{i-1})g(r_i), \tag{5}$$

where ϕ_i is the angle between the ith step $\mathbf{r}_i = (\phi_i, r_i)$ and the x-axis (of course, $\theta_{i+1} = \phi_{i+1} - \phi_i$).

The correlated walk model predicts (Hall, 1977, Eq. 12) the dimensionless quantity $E(R_n^2)/E(r^2)$ as a function of n as follows:

$$E(R_n^2)/E(r^2) = s(n - b) + sb\lambda^n, \tag{6}$$

where $\lambda = E(\cos\theta)$ is a measure of the degree of correlation between steps, and s and b are the following explicit functions of λ:

$$s = 1 + 2\lambda a/(1 - \lambda), \quad b = 2\lambda a\{(1 - \lambda)(1 - \lambda + 2a\lambda)\}^{-1}, \quad \text{and} \quad a = E(r)^2/E(r^2).$$

Thus, once the ratio a is found from the step-length data, and $\lambda = E(\cos\theta) \approx \overline{\lambda}$ is found from the empirical θ histogram, the prediction (Eq. 6) of the model is completely determined: there are no other adjustable parameters.

In the case of a random walk, $\lambda = 0$ and we have from Eq. 6 $E(R_n^2)/E(r^2) = n$, i.e. a straight line with slope 1; in general (except for some other very special cases that also yield the random-walk result, see Hall, 1977, section 2) $0 < \lambda < 1$ and Eq. 6 is curved upward and asymptotic to the straight line $y = s(n - b)$. For amebae we found $\lambda \approx 0.7$, whereas for granulocytes $\lambda \approx 0.3$; the corresponding slopes s of the asymptotes are consequently $s = 4.5$ and $s = 1.8$: the correlated walkers drift farther than a random walker.

The parameters whose values we need to test the predictions of the model against the experimental results have been estimated from the totality of raw data (rather than from the grouped data of Figs. 2 and 3) and these values are shown in Table I. The corresponding values obtained from the densities 2.2 and 2.3 are also shown, although we do not use the

TABLE I
PARAMETERS ESTIMATED FROM THE TOTALITY OF RAW DATA

Parameters	Data	Densities
$\lambda = E(\cos\theta)$	0.33	0.33
$\mu_r = E(r), \mu m$	14.9	15.0
$\sigma_r = (E(r-\mu_r)^2)^{1/2}, \mu m$	8.0	7.8
$a = E(r)^2/E(r^2)$	0.78	$\pi/4 \approx 0.785$
$b = 2\lambda a(1-\lambda)^{-1}(1-\lambda+2a\lambda)^{-1}$	0.65	0.65
$s = 1 + 2\lambda a(1-\lambda)^{-1}$	1.77	1.77

densities explicitly in what follows; they only provide a summary of the data within the terms of the model and could of course be used to simulate trajectories of the type we have studied.

In Fig. 4 we plot Eq. 6 using the parameter values given in Table I. These values were obtained from the trajectories of 13 cells with n between 24 and 49. Although the correlation between t (time) and n (step number) is not simple, cells that only made 24 steps before ceasing movement are considered to be older than cells that made a large number of steps during the observation period; that is, cells that made many steps during the observation period started out as young cells and aged during the course of the experiment. For $n > 30$ cell movement slowed visually, and although the cells continued to move, their movements consisted of short steps without a substantial net displacement. When these movements ceased, we concluded that the cell had died.

The theoretical curve shown in Fig. 4 agrees well with the experimental data for $n < 15$. For $15 < n \leq 30$, the experimental data increase faster than the theoretical curve. For $n > 30$, $E(R_n^2)$, is essentially constant and represents the epoch when the cells make short steps with little net displacement (senescence). The deviation of the experimental data from the theoretical curve is interesting in terms of the model. The curvilinear upward trend in the

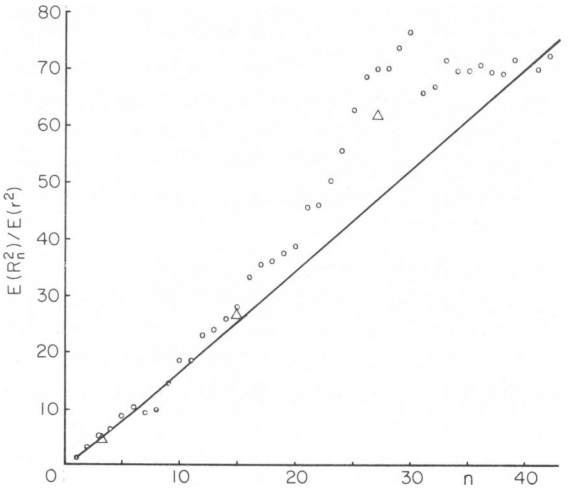

FIGURE 4 Empirical estimates o of $E(R_n^2)/E(r^2)$ from only the seven cells that made more than 40 steps against n; and the theoretical curve Eq. 6. Δ, theoretical points for three local fits to the data.

empirical data cannot be explained by the model with a and λ fixed. Hall (1977) has examined the consequences of λ varying between 0 for a random walk and 1 for a highly correlated walk. As λ becomes larger, the walker will move further from the starting point than a walker with a smaller λ. The empirical data for the granulocytes seems to suggest that λ changes during the course of the experiment. To test this hypothesis we have selected the intervals $n(1\text{–}5)$, $n(12\text{–}17)$, and $n(25\text{–}29)$ and computed new values of λ for cells in each interval. The data available for these computations is scant (35 angles per interval) and consequently the corresponding values of λ must be considered tentative, i.e. $\lambda = 0.36$, 0.38, and 0.47, respectively. The triangles on Fig. 4 represent new calculated values for $E(R_n^2)/E(r^2)$ for each of the above λ's and for $a = 0.78$. Those theoretical points fitted locally agree better with the experimental data and this suggests to us the following tentative explanation: as the cells age, they tend to turn less, and consequently λ and the slope s increase; later the cells also move very little so that the value of $E(R_n^2)/E(r^2)$ for $n > 30$ remains approximately constant.

As we have stressed above, n is not related to time in a simple way, but our work to date suggests that we should now investigate the variation of λ with time to see if this parameter (representing the degree of correlation) does vary consistently as the cells age. The purpose of this work is to try to build a reliable and consistent model for neutral motion before we approach the more complicated situation of chemotaxis.

DISCUSSION

We have characterized the spatial trajectories of human granulocytes moving in two dimensions under isotropic conditions as realizations of a correlated walk. This representation, also found satisfactory for the trajectories of slime-mold amebae (Hall, 1977), requires the estimation of three basic parameters, namely λ, μ_r, and σ_r. For comparison between different cells and between the same cells on different surfaces, the parameters λ, s, the slope of the asymptote to the theoretical curve $(E(R_n^2)/E(r^2), n)$, and μ_r appear to be the most useful: s is sensitive to the degree of correlation λ and has the value $s = 1$ for a random walk; μ_r indicates the scale of the trajectories. We compare the two types of cell (both moving on glass) that we have studied to date in Table II. For close comparisons, rather more data would be required to obtain the necessary accuracy for the parameters.

We have assumed that the density of the cells is sufficiently low that cell-cell interactions can safely be ignored. Both the amebae and the granulocytes were found to cross their own and each other's paths and we suspect that this phenomenon, which we have not taken into account, may be important.

This current model describes the movements of human granulocytes in much more detail than our previous one (Peterson and Noble, 1972). The parameters μ_r, s, and λ can be

TABLE II
COMPARISON OF TWO TYPES OF CELL MOVING ON GLASS

	Shape of $f(\theta)$	λ	s	μ_r
				μm
Slime-mold amebae	Unimodal	0.7	4.5	24
Human granulocytes	Bimodal	0.3	1.8	14.5

obtained for cells moving under different experimental conditions. These parameters should allow a more sensitive comparison between cells undergoing "normal" movement and those undergoing chemokinesis or chemotaxis.

Unimodal turn angle distributions have been reported for horse polymorphonuclear leukocytes (PMNs) (Nossal and Zigmond, 1976) and human neutrophils (Allan and Wilkinson, 1978). In both cases, the preparative techniques used, and in the latter case the requirement for casein, differ from our method. In both investigations cited above, PMNs were subjected to sedimentation, anticoagulants, and either albumin or Ficoll-Triosil gradients. Our less complicated preparation may represent the basis for the different turn angle distribution reported here. If indeed different preparative techniques alter the turn angle distribution made by PMNs and if the turn angle distribution is important in the chemotactic response of the cells, as we suspect, future studies of PMN locomotion and response to stimuli must be carefully interpreted in terms of the particular technique used to prepare the cells.

Preliminary investigation of human granulocytes undergoing chemotaxis indicates that the parameters s and λ (estimated locally) both increase as the cells approach the attractant at a rate greater than in the isotropic case. However, the relative angle θ still appears to be statistically independent of the step length r, which increases slowly with increasing n. As Zigmond (1978) points out, PMNs undergoing chemotaxis can start out, for example, in the direction of the gradient, and the step angle ϕ (to a fixed axis) may in this case not be randomly distributed. If this is indeed the situation, then our model may have to be altered to take this into account.

The biological basis of the increase in λ with epoch n must remain speculative at this time, since we do not know which cellular mechanism is responsible for the distribution of angles between steps. If the ability of a cell to turn through various angles depends on inherent structures, such as bundles of microfilaments, then the bundles may become less dispersed as n increases. Alternatively, a sensory control mechanism involved in ordering the turns may eventually become less able to direct large angular changes.

If, as our preliminary results suggest, λ increases during chemotaxis, chemotaxis may simply consist of a more highly correlated walk than when movement occurs under isotropic conditions. Nossal and Zigmond (1976) have reported just the opposite. They find that the turn angle distribution becomes bimodal when the cells are undergoing chemotaxis (1976); find a unimodal distribution for θ under isotropic conditions (their Fig. 3), and find a bimodal distribution when the cells are undergoing chemotaxis (the graphs on their Fig. 2*b* added to the reflections in the $\theta = 0$ axis). Using the data from these figures, we find that the parameter λ of our theory has the value $\lambda = 0.8$, approximately, for all four graphs of Nossal and Zigmond's paper. However, these authors exhibit rather few data points and they have omitted data for direction angles $\phi > 45°$.

We hope that the model presented here will stimulate careful comparison of the characteristics of PMN movement under different conditions. Such comparisons are clearly needed.

The authors would like to thank Dr. P. Noble for his generosity with the data and his useful comment.

This work was supported in part by a Medical Research Council of Canada grant MA 5340.

Received for publication 15 June 1978 and in revised form 25 September 1978.

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