A TWO-DIMENSIONAL RANDOM-WALK ANALYSIS OF HUMAN GRANULOCYTE MOVEMENT

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ABSTRACT An equation describing a two-dimensional random walk with nonuniform step length is derived and applied to a cinephotomicrographic analysis of human granulocyte movement. The method is also applied to a generally recognized chemotactic movement.

INTRODUCTION

Chemotaxis is an important phenomenon relevant to many areas of physiology and developmental biology. With one exception (1), methods of assessment of chemotaxis are largely subjective (2–4). We were interested in finding a technique which would be applicable to cinephotomicrographic analysis of cell movement and which would allow us to determine through statistical analysis whether cells were moving randomly or whether they were under the influence of external forces. Two-dimensional random-walk theory applied to cell movement would allow us to predict the most probable distance that a cell would be from its starting point if its motion were random. We were, however, unable to find an easily derived random-walk equation for nonuniform step size. The following derivation is relatively simple, and the resulting equation is applicable to cinephotomicrographic studies. Since random-walk theory is itself probabalistic the movement of many cells under identical conditions must be studied and analyzed statistically. We present two examples of the application of the method. First we consider the movement of granulocytes with no apparent external forces acting on them, and second, the same kind of cells moving towards a concentration of bacteria, a generally recognized example of chemotaxis.

THEORY

As will be shown below the most probable distance which a randomly moving particle with nonuniform step length moving in two dimensions will be from the starting point after n steps is given by:

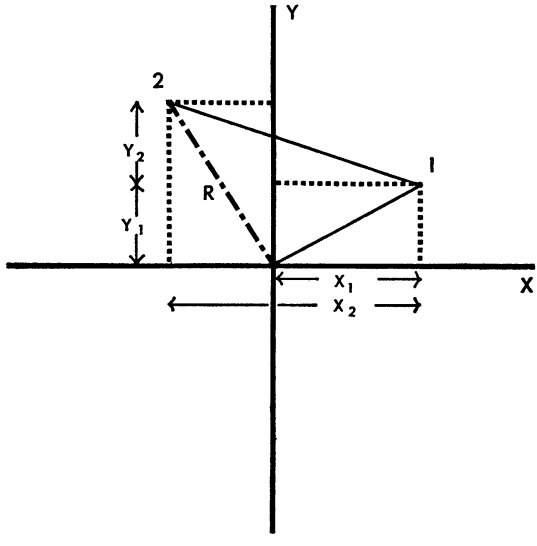

FIGURE 1 Coordinate system and nomenclature used in describing a "two-step" random walk.

$$R = \left[\sum_{i=1}^{n} s_i^2\right]^{1/2}, \tag{1}$$

where R is the most probable distance after n steps of length s_i.

The following derivation of equation 1 is similar to Gamow's (5) but arrives at a substantially different result. Let the starting point for movement be the origin of a two-dimensional coordinate system (Fig. 1). Let the particle take a first step of random length (s_1) and direction to point 1. The projections of s_1 on the x and y axes are x_1 and y_1. If the particle takes another step of random length s_2 in another random direction the new coordinates will be given by:

$$X = x_1 + x_2,$$

where x_2 is the projection of s_2 on the x axis. Similarly $Y = y_1 + y_2$. The final distance R after n such steps is given by:

$$R^2 = X^2 + Y^2,$$

where $X^2 = (x_1 + x_2 + x_3 + \cdots + x_n)^2$ and $Y^2 = (y_1 + y_2 + y_3 + \cdots + y_n)^2$, or $R^2 = (x_1^2 + x_1x_2 + x_1x_3 + \cdots + x_2^2 + x_1x_2 + x_1x_3 + \cdots + x_n^2) + (y_1^2 + y_1y_2 + y_1y_3 + \cdots + y_2^2 + y_1y_2 + y_1y_3 + \cdots + y_n^2)$. Now for large n there will be about as many negative x as positive of equal magnitude so that, on the average, positive mixed products will have a negative counterpart and these will cancel; the same is true for y. Therefore we are left with

$$R^2 = x_1^2 + x_2^2 + x_3^2 + \cdots + x_n^2 + y_1^2 + y_2^2 + y_3^2 + \cdots + y_n^2.$$

Regrouping, we obtain:

$$R^2 = x_1^2 + y_1^2 + x_2^2 + y_2^2 + x_3^2 + y_3^2 + \cdots + x_n^2 + y_n^2.$$

Now for any given step s_i it is obvious that

$$s_i^2 = x_i^2 + y_i^2,$$

so that

$$R^2 = s_1^2 + s_2^2 + s_3^2 + \cdots + s_n^2, \tag{2}$$

or more compactly

$$R = \left[\sum_{i=1}^{n} s_i^2\right]^{1/2}. \tag{1}$$

Equation 1 thus represents the general equation for the most probable distance from the origin for a two-dimensional random walk.

If the length of the steps is the same, that is $s_1 = s_2 = s_3 \cdots$, then equation 1 reduces to:

$$R = [nS^2]^{1/2}. \tag{3}$$

This is the equation for a two-dimensional random walk of uniform step size (see, for example, Brown [2]). Equation 1 can also be written in terms of the mean step length $\bar{s}$ and the variance $\sum_{i=1}^{n} (\delta s_i^2/n)$ as follows. We can write any given step s_i as the mean step length $\bar{s}$ and the deviation δs_i:

$$s_i = \bar{s} + \delta s_i,$$

substituting into equation 2,

$$\begin{aligned} R^2 &= (\bar{s} + \delta s_1)^2 + (\bar{s} + \delta s_2)^2 + \cdots + (\bar{s} + \delta s_n)^2 \\ &= \bar{s}^2 + 2\bar{s}\delta s_1 + \delta s_1^2 + \bar{s}^2 + 2\bar{s}\delta s_2 + \delta s_2^2 + \cdots + \bar{s}^2 + 2\bar{s}\delta s_n + \delta s_n^2, \end{aligned}$$

the terms

$$2\bar{s} \sum_{i=1}^{n} \delta s_1 = 0,$$

since

$$\sum_{i=1}^{n} \delta s_i = 0;$$

thus

$$R^2 = n\bar{s}^2 + \sum_{i=1}^{n} \delta s_1^2,$$

or

$$R = n^{1/2}(\bar{s}^2 + \sum_{i=1}^{n} \delta s_1^2/n)^{1/2}. \quad (4)$$

MATERIALS AND METHODS

Preparation of Granulocytes

A drop of human blood, obtained from a pricked finger, was placed on a cover slip and kept in a covered Petri dish saturated with water vapor at 37°C for 30 min. After this time the blood clot was carefully removed and erythrocytes gently washed from the cover slip with Hanks' balanced salt solution. Actual counts showed that approximately 95% of the adhering cells remaining were granulocytes (neutrophils, basophils, and eosinophils).

Demonstration of Chemotaxis

The cover slip containing the cells was fastened with wax to one side of a Mackaness chamber, and a small length of capillary tubing containing *Staphylococcus aureus* was placed on the cover slip as shown in Fig. 2. In all experiments the chambers were filled with a tissue culture medium of the following composition: Grand Island Biological Co. (Grand Island, N. Y.), Minimum Essential Medium with Hanks' salts, L-glutamine (0.2 mmoles/100 ml), 20% fetal calf serum (heat inactivated) buffered to pH 7.4 with 25 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES). Control experiments were carried out in the same manner with the exception that the capillary tube containing *S. aureus* was omitted.

The paths taken by the cells were recorded using a Wild M40 inverted microscope and Wild time-lapse unit (Wild Heerbrugg Instruments Inc., Farmingdale, N. Y.). A total magnification of × 150 was used and one frame was exposed every 8 sec. At this filming rate cells did not move more than two cell diameters between frames. The chambers were maintained at 37°C using a Sage air curtain (Sage Instruments Div., Orion Research, Inc., Cambridge, Mass.).

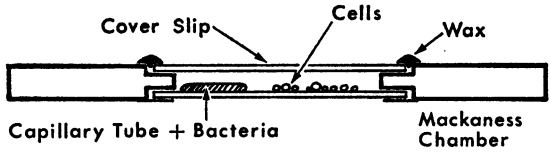

FIGURE 2 Side view of a Mackaness chamber used to study cell movement. The cells are photographed from below using an inverted microscope.

Analysis of Film: Determination of Step Length

Using a 16 mm analytical projector, the film was projected onto a white screen and the paths taken by the cells plotted. The step length was taken as the distance, in millimeters, between two successive changes in cell direction, for constant frame magnification (cf. Figs. 3 *a* and 3 *b*). We estimate that the minimum deviation from a straight line path that would be detected as a change in direction was 8°. From the cell paths plotted the direct final distance of the cell from point of origin, the number of steps taken, and the individual step length were determined.

RESULTS

Figs. 3 *a* and 3 *b* show typical examples of granulocyte movement under two different conditions. In Fig. 3 *a* the path taken by a granulocyte moving in the absence of bacteria is shown; in Fig. 3 *b* movement occurs in the presence of bacteria which were placed in a capillary tube as indicated. Table I shows the predicted (most probable) and actual distances that 16 cells moved in the absence of bacteria.

By using a paired two-tailed *t* test one can show that there is no significant dif-

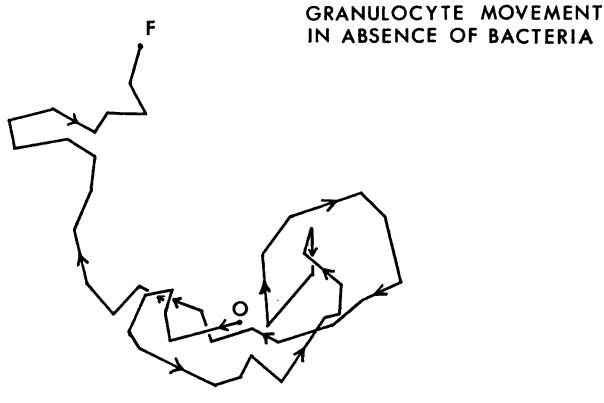

FIGURE 3 *a* The path taken by cell 7 in Table I. Movement starts at *O* and ends at *F*. Each straight line segment constitutes a step. The straight line distance between *O* and *F* approximately equals that predicted by random-walk theory.

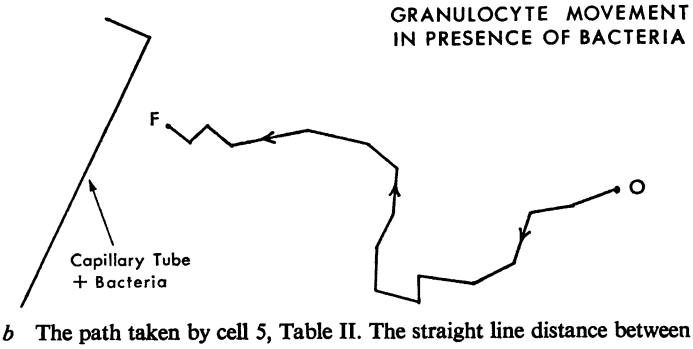

FIGURE 3 *b* The path taken by cell 5, Table II. The straight line distance between *O* and *F* exceeds that predicted by random-walk theory.

TABLE I

GRANULOCYTE MOVEMENT IN THE ABSENCE OF BACTERIA*

Cell No.	No. of steps	Predicted distance‡	Actual distance
1	48	69.1	60
2	14	40.0	93
3	27	59.5	41
4	33	54.4	67
5	21	37.2	20
6	43	76.9	220
7	43	68.8	66
8	19	35.0	16
9	36	58.1	15
10	47	62.2	161
11	35	51.2	64
12	74	88.3	161
13	18	33.0	79
14	27	50.4	57
15	24	43.7	74
16	21	40.9	47

* t paired = -1.898; $n = 16$.
‡ Computed from equation 1.

TABLE II

GRANULOCYTE MOVEMENT IN THE PRESENCE OF BACTERIA*

Cell No.	No. of steps	Predicted distance‡	Actual distance
1	27	46.8	159
2	9	23.8	42
3	19	48.8	172
4	8	45.7	101
5	16	40.2	102
6	17	50.4	136
7	11	37.3	85
8	19	49.2	164
9	8	34.5	62
10	28	58.8	201
11	9	35.4	86
12	19	50.3	118

* t paired = -6.59; $n = 12$.
‡ Computed from equation 1.

ference ($P > 0.05$) between the actual distance and the predicted distance the cell would be from the starting point if its motion were random. Thus granulocyte movement in the absence of bacteria conforms to our equation for random motion.

Table II summarizes the movement of granulocytes in the presence of bacteria,

an accepted chemotactic phenomenon. Comparison of actual and predicted distances by a paired two-tailed t test indicates a highly significant difference between distances ($P < 0.001$). The movement of granulocytes in the presence of bacteria does not conform to random-walk theory.

DISCUSSION

The theoretical basis for the random-walk equation presented here is quite straightforward. A key point in the derivation is the determination of the step length. From a theoretical point of view the step length is the straight line path between two successive changes in direction. One cannot, for example, divide such a step into smaller segments and treat these segments as individual steps since the second, third, etc., steps are thus predetermined and no longer random. From an experimental point of view this prohibition rules out using the change in position of a particle after an arbitrary number of photographic frames (length of time) as a measure of step length.

One difficulty which arises is in trying to decide whether a cell has in fact changed the direction of its movement. From a practical point of view most steps are easy to assign, but there will be cases when there are only very slight changes in direction and some error will be incurred in deciding whether a change has in fact occurred. The error that is most likely to occur is an underestimate of the number of steps and an overestimation of step length. If, for example, two actual steps (a and b) are treated as one large one, R^2 will be overestimated by ($2ab$). Since an overestimate of R occurs one would predict that the cell would move farther from the origin than if the error were not made. This in turn would, for example, give an underestimate of positive chemotaxis. In our system we can detect variations in direction as small as 8°. There are thus 45 8°-segments into which a cell can move for any given random step. The probability that a cell will take a second random step in the same segmental direction is $1/(45)^2$ or 1 chance in 2025. If the cell is moving randomly the probability that it will take two successive steps in the same direction is relatively small, and estimates of R should be reasonably accurate using the method presented in this paper. It is wise, however, to consider the effect of such errors on the experimental conclusions.

The random-walk technique presented in this paper allows statistical analysis of cell movements to be made. While it cannot be stated categorically that the motion of granulocytes is not under the influence of external forces, their motion is statistically indistinguishable ($P > 0.05$) from random motion. The movement of granulocytes toward a concentration of bacteria is statistically distinguishable ($P < 0.001$) from random motion. The random-walk technique should prove itself useful in cases where chemotaxis is not as apparent as in the example presented here. Obviously if the departure from random motion is not as great as in the above example, a larger sample of cells would have to be studied to produce the necessary statistical assurances.

With this technique it is also possible to get comparisons of departure from randomness. For example, the ratio of the mean actual distance moved over the mean predicted distance can be calculated. Ratios of departure can be computed for different experimental conditions and, for example, the effectiveness of different chemotactic agents compared. Similarly dose-response curves can be constructed.

Boyden's quantitative method for assessing chemotaxis, using a Millipore filter (Millipore Corp., Bedford, Mass.) to partition cells from chemotactic substances, does not readily lend itself to the analysis of those aspects of cell behavior which lead to aggregretion, sorting out, and organ histogenesis (1). We feel that the application of the random-walk technique as described here will help to resolve events leading to aggregation which have been stated as being "random" without any quantitative test for randomness being made (6–9).

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