The Response of Human Neutrophils to a Chemotactic Tripeptide
(N-Formyl-Methionyl-Leucyl-Phenylalanine)
Studied by Microcinematography

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A quantitative microcinematographic technique for the measurement of cellular motion in response to chemical influences is described. The data are analyzed by using a magnetic digitizer connected to a computer, permitting comparison of several characteristics of such motion. When human granulocytes are placed in a gradient of formyl-methionyl-leucyl-phenylalanine (fMLP) (concentration 10^{-8} - 10^{-6} mol/L) against buffer, the motion of the cells is increased in velocity (chemokinesis) and is directed toward the chemoattractant, as measured by the orientation of the vector of motion. This directionality is confirmed by positive values for the McCutcheon index and analysis of directed linear displacement. Concentrations of fMLP below 10^{-8} mol/L did not result in chemokinesis or chemotaxis, presumably due to insufficient stimulus. Concentrations of 10^{-6} mol/L fMLP and greater resulted in marked stimulation of the cell surface but reduced directionality and velocity of motion compared with cells in a more optimal gradient. Motion of the unstimulated cells tended to be ortholinear, and this was not increased by the presence of a gradient of fMLP. Hence, as previously shown, the Random Walk analysis, which does not give weight to the direction of motion, is not useful in the analysis of the response of these cells to a chemotaxin in this system.

CHEMOTAXIS, the directed migration of cells in a chemical gradient, was first observed and measured visually using repeated still photographs or microcinematography. In these studies, clumps of microorganisms served as point sources of chemoattractant. Although there has recently been increased interest in such techniques, these visual techniques are difficult to use for three reasons: (1) the methods employed are difficult to adapt to the study of soluble chemotaxins, (2) satisfactory chemical gradients are difficult to establish and maintain on a microscope slide, and (3) data collection for quantitative analysis is difficult and time consuming. Because of these limitations, chemotaxis has often been measured in Boyden chambers, in which a chemoattractant was placed on one side of a filter and migratory cells on the other. The degree of attraction is measured by enumerating the number of cells entering or penetrating through the membrane toward the chemoattractant. The Boyden chamber has the advantages of being a rapid and, with new techniques of data analysis, an accurate assay system. However, it is not possible to analyze the chemotactic activity of individual cells nor to determine easily whether populations of cells exist that differ in their chemotactic activity. Furthermore, it is difficult to distinguish chemotaxis from chemokinesis (stimulation of motion without regard to orientation).

Recently, Zigmond has designed a chamber that permits the formation of a very stable gradient and, at the same time, permits the observation of cells in that gradient. Initial studies using the chamber measured neutrophil orientation in response to N-formyl-methionyl-leucyl-phenylalanine (fMLP), but did not analyze cell motion. This tripeptide had previously been shown to induce chemotaxis of human neutrophils using the Boyden chamber assay.12-15 Saturable, high-affinity receptors for formyl peptides have been identified on the human neutrophil plasma membrane using radioactive14,15 and fluorescent16 ligands. In this article, we assess the chemotactic and chemokinetic effects of fMLP on human neutrophils using time-lapse cinematography to measure both orientation and locomotion. The data collected in this way have been analyzed using a computer system, and several mathematical models of analysis have been compared.

MATERIALS AND METHODS

Materials

Dextran solution was composed of 2% Dextran (mol wt 170,000) dissolved in normal saline (Pharmacia Fine Chemicals, Piscataway, NJ). Ficoll-Hypaque consisted of 9% Ficoll (Sigma Chemical Co, St Louis, Mo) mixed with 30% Hypaque (Winthrop Laboratories, New York, NY) and adjusted to a specific gravity of 1.077 at 4°C with distilled water. Buffer for cell suspension was Gey's balanced salt solution with 2% bovine serum albumin (GBSS + 2% BSA) (Flow Laboratories, Rockville, Md). The chemotaxin, N-formyl-methionyl-leucyl-phenylalanine (courtesy of Dr S. Wilkinson, Burroughs-Wellcome, Beckenham, England), was a stock solution of 10^{-3} mol/L in ethanol, diluted in buffer to working concentration as necessary.

Chemotactic Chamber

The observation chamber, designed after that of Zigmond,8 consisted of a plexiglass slide measuring 2 inches in length, 22 mm in...
width, and \( \frac{1}{2} \) inch in depth. Two troughs, 1 mm apart, were cut across the width of the slide at its center. This created two wells (100 
\( \mu \)L vol) separated by a central bridge; when a coverslip was placed across the chamber, a thin film of fluid, 3-10 \( \mu \)m deep, was formed over this bridge. It has been shown that a gradient is formed across the bridge within 1 minute and is steep and stable between 15 and 90 minutes.4

**Photographic Equipment**

Films were taken with an Arriflex 16S motion picture camera equipped with a DOM motor and stop-motion console, attached to an Arriflex Master Intervalometer, model 12, on a Zeiss Universal Transmitted Light Microscope (Karl Zeiss, Oberkochen, West Germany). Pictures were taken with Nomarski optics at a magnification of \( \times50.5 \). The microscope stage was maintained at 37°C by a Sage Air Curtain Incubator (Orion Instruments, Cambridge, Mass).

**Methods**

Human polymorphonuclear neutrophils (PMNs) were obtained from 5 mL heparinized whole blood from healthy donors by sedimentation with an equal volume of 2% Dextran in normal saline for 45-60 minutes. The supernatant suspension was layered on a discontinuous Ficoll-Hypaque gradient and centrifuged for 20 minutes at 2,000 rpm.17 The pellet was resuspended in 5 mL hypotonic lysing solution [2.06% Tris base, pH 7.2; 0.83% \( \text{NH}_4\text{Cl} \) (1.9 vol/vol)] and incubated at 37°C for 10 minutes to lyse the red blood cells. The purified PMNs were washed three times in Gey's balanced salt solution with 2% bovine serum albumin and diluted to an approximate concentration of \( 4 \times 10^7 \) cells/mL. Final cell suspensions contained 95% PMNs and were kept at 37°C for the duration of the experiment.

PMNs were allowed to adhere to a hemacytometer cover slip (Scientific Products, 0.4-mm thickness) for 5-10 minutes in a 37°C humidified chamber. The slide was then washed three times with warm buffer and inverted over the observation chamber containing 100x of GBSS + 2% BSA in each well. For observation of stimulated motion, one well was evacuated with a tuberculin syringe and refilled with IOOX of a solution containing the appropriate concentration of FMLP dissolved in GBSS + 2% BSA.

Black-and-white films (Kodak Plus-X Reversal 16-mm black-and-white film) were made at a rate of one frame every 4 seconds for a period of 20-23 minutes. The camera was focused as closely as possible on the center of the chamber bridge, and filming was begun directly after instillation of the chemotaxin. A standard grid was photographed with each sequence for calibration of the distances measured.

**Data Collection**

A Lafayette Analyzer Projector (Lafayette Instruments, Lafayette, Ind) was used to project the film sequences onto a sheet of graph paper that had been calibrated against the reference grid. The long axis of the chamber bridge was aligned with the y-axis, and the chemotactic well was taken to be at infinite distance on the x-axis so that each cell could be analyzed in its own x-y coordinate system. The center (estimated visually) of each cell on the film at 13.3 minutes (frame 250) was plotted and assigned the value (0.0). Unless otherwise noted, the center of each cell was successively plotted every 40 seconds (10 frames) for a total of 400 seconds, or until the cell left the visual field. This resulted in a maximum of 10 data points for each cell. If additional time intervals were studied, plotting was carried out in the same manner for each subsequent 400-second interval. Paths between data points were assumed to be linear, and cells that did not move at all during the time period considered were eliminated from further calculation (less than 10%). The visual field measured 300 \( \times \) 235 \( \mu \)m, and the number of cells per field during a given analysis varied from 9 to 35, with an average of 15.

**Computer Measurement of Microcinematographic Data**

The program for acquiring and analyzing the chemotaxis data was written in a PL/1 language subset, which runs on the Digital Equipment Corporation PDP-11 series computers under the RSX-11D or IAS operating systems. Data were entered with a magnetic digitizer (Summagraphics, Fairfield, Conn) and displayed on a vector graphic terminal (Tektronix). The four phases of the program were data collection, retrieval, display, and analysis.

In the data collection phase, the plot of the cell tracks was placed on the digitizer pad, and reference points and cell locations were rapidly entered by placing the crosshairs of the hand unit over the appropriate position and pressing a button, thereby transmitting the Cartesian coordinates to the computer. Scale parameters (two points on the date sheet that are 50 \( \mu \)m apart) were entered for the group of cells. For each cell to be entered, boundary lines were defined so that partial data concerning cells that travel out of the field could be entered. The location of the cell at fixed intervals was then recorded. As the points were defined, they were graphed on the display for visual verification, and at the conclusion of data entry of each cell, optional repetition of the data entry was permitted. The computer automatically performed a coordinate rotation on the data so that all cells were stored with the site of chemotaxin at 0° in a polar coordinate system.

Once the data were satisfactorily entered, they could be stored in a data file for subsequent retrieval, display, and analysis. The display phase allowed the operator to review the data in several forms. For each cell, the x and y coordinates of each position on the path could be displayed. Alternatively, the path could be graphed with an adjacent table, giving the angle of each step relative to the direction of the preceding step, and the corresponding length in microns (Fig 1). A graph of the paths of all cells in an experiment could be displayed simultaneously to the same scale, giving an indication of

![Fig 1](https://example.com/fig1.png)
the uniformity of chemokinesis, or could be normalized to the same display size with the magnification factor displayed by each cell, revealing the chemotactic response more clearly.

The analysis routine provided the calculations necessary to determine the parameters of interest for each cell and a tabulation of the cell population indices. These data manipulations were the most tedious part of an experiment, and the most error-prone when done manually. Values calculated included, for each cell step, the angle of direction change and step length, and for each cell path, the total path length, radial direction of travel, McCutcheon index, average velocity, and effective velocity.

Analysis of Cellular Responses to fMLP

**Cellular Velocity**

The linear velocity of each cell was computed from the mean for each cell.

**Orientation of Motion**

The angle of vector describing the motion of a cell during each interval of 40 seconds was computed; motion toward the right-hand well was assigned the value of 0°. Motion perpendicular to this vector was assigned the values of +90° or -90°. Motion toward the left-hand well was assigned the value of 180°.

**Cellular Tracking**

The degree to which a cell deviated from linear motion in successive intervals was determined by measuring the change in angle of the vector in successive steps. The frequency with which this change in angle fell within defined limits was determined; e.g., if the arbitrary limit of angular change (θ) was taken at 25°, the frequency was determined by dividing the number of steps in which the angle was ≤25° by the total number of interval steps. A value of 1.0 indicated that the angular change of all steps was within the assigned values.

**Measures of Chemotaxis**

The tendency of a cell to travel toward a chemotactic stimulus was measured using several methods of analysis.

**The McCutcheon index.** The McCutcheon index (chemotactic ratio) identified the proportion of each cell path traversed in the designated direction. It was calculated as the ratio of the length of the cell path traveled directly toward the chemotaxin to the length of the total path. As the attractant emanated from a line source situated at “infinity” on the x-axis, the McCutcheon index could be calculated as the length of the projection of the cell path on the x-axis divided by the total path length. Positive values were assigned to projections toward the chemotactant, negative values to those away from it. Thus, a cell travelling directly toward the stimulus would have an index value of +1.0, and a cell travelling directly away from the stimulus would have an index value of -1.0. This measure is normalized for path length and velocity and, thus, was comparable for all cells regardless of speed or length of time observed.

**Directed displacement analysis (stimulated Boyden chamber).** The net linear displacement of each cell from the origin toward or away from the chemotactant was measured as the projection of the total path length for 400 seconds on the x-axis. For cells observed less than 400 seconds, this distance was normalized to that time.

**Random Walk analysis.** Analysis by the Random Walk theory was applied to both stimulated and unstimulated populations of cells. In this analysis, the most probable distance travelled in successive steps from the origin was calculated, assuming that directionality was not dictated, using the following equation:

\[ R = \left[ \sum_{i=1}^{N} S_i \right]^{1/2} \]

where \( R \) = the most probable distance travelled after \( N \) steps of length \( S \). The length of step in this analysis was taken as the distance travelled without a change in angle greater than some arbitrary amount. Peterson and Noble used 8° as the angle of turning that defined the end of a step; we used 10°. If the observed distance travelled from the origin \( (O) \) was less than the distance predicted by the equation \( (P) \), the motion was nonrandom; if greater, the motion was random. The \( O/P \) ratio was calculated for each cell.

**RESULTS**

To determine the effect of varying the length of the time interval over which the velocity was calculated on the apparent velocity, the same films were analyzed using intervals from 12 to 60 seconds to calculate the velocity of the cells. With diminution of the interval, the possibility of a cell turning during that interval decreased, therefore, the apparent (calculated) velocity increased (Fig 2); this was observed for both unstimulated and stimulated cells. However, the apparent mean velocity was the same for intervals of 5 seconds, 10 seconds, and 12 seconds (data not shown). As a matter of convenience in data collection, the 10-second interval was used for all data unless otherwise stated.

The average cellular velocity of unstimulated cells was 7.4 μm/min, with a range of 2.5 to 17.3 (Fig 3). Velocity was significantly increased \( (P < .01) \) in gradients formed with buffer against \( 10^{-6} - 10^{-8} \) mol/L fMLP. The maximal mean velocity was 21.5 μm/min at \( 10^{-7} \) fMLP and decreased to baseline levels at both...
higher (10⁻⁵ mol/L) and lower (10⁻¹⁰ mol/L) concentrations of chemotaxin. In gradients established between a concentration of fMLP and a 100-fold dilution of that concentration, maximal velocity was 17.6 µm/min and velocity remained significantly elevated at 10⁻¹⁰ mol/L fMLP. Because time spent motionless would alter the calculated average velocity, the proportion of observed intervals during which no translation occurred was measured for cells at each concentration of the gradient (Table 1). Neutrophils were almost never stationary, whether or not a gradient was present. Hence, the calculated average velocity was not significantly affected by inclusion of all data. Totally immobile cells were not included in any other analysis.

Detailed analysis of the velocity of cells without chemotactic stimulus and in a gradient of 10⁻⁷ mol/L fMLP v buffer is shown in Fig 4. The velocity of undivided steps of unstimulated cells resulted in an asymmetric distribution about the mean; there appeared to be a virtual lower limit of velocity, and a small number of steps were taken at velocities more rapid than usual, resulting in a skew of the curve. In a chemotactic gradient, the mean velocity was increased and the asymmetry of the curve was reversed. There appeared to be an upper limit of the observed velocity, and some steps were taken at velocities less than the mean, resulting in a skew of the curve of the lower velocities.

The vector angle of motion was determined for each
Concentration of fMet-Leu-Phe (Molar)

Fig 7. The McCutcheon index (chemotaxis index) as a function of the concentration of fMLP used in forming a gradient. Gradients were formed between the concentration given and either GBSS + BSA (shown in solid figures) or a 100-fold dilution of the given concentration of fMLP (shown in open figures). The mean and SEM of the indicated number of determinations on multiple runs is shown.

successive interval by reference to the x-y coordinate system described above. Changes in the vector angle in successive intervals were then calculated to determine the frequency with which cells changed direction during movement. Cells undergoing undirected ("random") motion tended to have limited change in vector angle over successive intervals; the frequency with which the angular change was less than 45° was high (76%) (Fig 5).

The orientation of movement with respect to the presence of a gradient of fMLP was measured by assessment of the angle of the displacement vector for each time interval. When no gradient was present, there was no preferred angle of orientation of movement (Fig 6). With increasing gradients, the angle of orientation of movement progressively approached 0° (directed toward the stimulus), with little variation at the optimum gradient (10⁻⁷ mol/L) (Fig 6). As the concentration of the chemotactant was increased above 10⁻⁷ mol/L, variability in the angle of orientation again occurred. Detailed analysis of cell movement demonstrated that the angle of orientation corresponded to the angle at which the lamellipod of the cell was oriented (data not shown).

As a measure of chemotaxis, the McCutcheon index was determined for cells in gradients formed between buffer and concentrations of fMLP ranging from 10⁻⁵ to 10⁻¹⁰ mol/L (Fig 7). Indices significantly different (P < .01) from that obtained with unstimulated cells were seen in gradients formed with 10⁻⁶, 10⁻⁷, and 10⁻⁸ mol/L fMLP. When the gradient was formed between the same concentrations of fMLP and concentrations 100-fold less, results were the same as those observed when the gradient was formed against buffer. When fMLP was added in equal concentration in both wells, the cells migrated to the nearest edge at increased velocity (the "edge effect" of Zigmond).

To assess the response using both velocity and direction, we calculated the directed mean displacement for each cell. Maximum displacement in the direction of the chemotactic gradient occurred at gradients made with 10⁻⁷ and 10⁻⁸ mol/L fMLP and was decreased at lower and higher concentrations (Fig 8). Although the distribution of displacement of unstimulated cells appeared to be normal about the mean at the origin, the distribution of displacements of stimulated cells was not normal about the mean and tended to have considerable skew. Consequently, these results were analyzed using nonparametric statistics (Mann-Whitney analysis).

When the Random Walk equation was used to analyze motion, the observed distance (D) exceeded the predicted distance (P) even in the case where no chemoattractant was present (mean O/P ratio was 1.79 ± 0.08) (Fig 9). None of the ratios calculated for cells in gradients of fMLP was significantly different from that calculated for unstimulated cells (P > .1).
The methods for measuring cell movement in response to chemical stimulants are, in general, of two sorts: (1) direct visualization of cell movement by photomicroscopy, and (2) indirect measurement of cellular migration through filters. The visual techniques, first used in 1932, are recognized as being the most direct and “physiologic,” but have not been widely used for several reasons. First, it is difficult to set up an adequate chemical gradient on the usual slide-coverglass preparations, except with particulate sources such as bacteria, yeast, or beads soaked in a chemotactic stimulus was assigned positive values; linear displacement away from that stimulus was assigned negative values.

**DISCUSSION**

The concentration of fMLP used in forming the gradient against GBSS + 2% BSA. Linear displacement in the direction of the chemotactic stimulus was assigned positive values; linear displacement away from that stimulus was assigned negative values.

Thus, the two major difficulties encountered in measuring chemotaxis by visual methods have been overcome.

It is clear from observation of the films taken of cells in gradients of fMLP ranging from $10^{-8}$ to $10^{-6}$ that directed movement toward the higher concentration of chemoattractant is occurring. Zigmond previously characterized orientation of cells in a gradient by observing that cellular lamellipods projected toward higher concentrations of chemotaxin. The present studies confirm that orientation of the lamellipod in a given direction indeed corresponds to migration of the cell in that direction. Cells undergo locomotion in the direction of projection of the lamellipod whether or not they are in a chemotactic gradient. Although unstimulated cells are able to change their direction frequently, they do not tend to change their direction in a strictly random fashion. That is, during each interval of observation, they tend to go in a direction not much different from the direction they pursued during the previous interval. For this reason, relative ortholinearity of the cell path was not a good measure of the effect of the chemotaxin upon locomotion.

The orientation of the lamellipod and, hence, the cellular motion in a chemical gradient, is thought to be the result of recognition by the cell of a differential occupancy of surface receptors for the chemotaxin across the cellular length. Based on this assumption, Zigmond has postulated that sensitivity for orientation would be maximal at the chemotaxin concentration that produces 50% receptor occupancy. At this concentration, the greatest relative change in receptor occupancy would occur for small changes in the chemotaxin concentration. Radioligand binding studies have demonstrated 50% receptor occupancy at 12–14 nmol/L and 30 nmol/L fMLP. Our data demonstrate maximal orientation between 10 and 100 nmol/L (Fig 5), which supports the differential occupancy hypothesis. The previous study, which used a visual assay, described maximal orientation between 1 and 10 nmol/L. However, orientation was simply assessed by assigning a positive value to cells whose lamellipods faced the attractant (ie, within a 180° arc) and a negative value to those that faced away. Using this criterion, we would find equal orientation at 1, 10, and 100 nmol/L.
At gradients against high concentrations of fMLP (10^{-3} \text{ mol/L}), directed motion and velocity were decreased. Cells in this concentration had very active lamellipod formation—much more active than that of unstimulated cells—but the lamellipods were oriented in all directions; hence, locomotion and, particularly directed locomotion, were decreased. This suggests that at such concentrations, differential occupancy of receptors was no longer present, and because receptors on all segments of the membrane were occupied, lamellipod formation occurred in all directions. Similar behavior of human neutrophils exposed to saturating levels of fMLP has been shown to correlate with increased adhesiveness and may be the mechanism for nonspecific deactivation of the chemotactic response.\textsuperscript{12,19}

Four techniques were used to analyze the directedness of motion in the chemical gradient: angle of orientation of motion, McCutcheon index, directed linear displacement, and Random Walk. Of these, two (McCutcheon index and directed linear displacement) proved useful and amenable to statistical analysis; the other two did not. Both the McCutcheon index and the directed linear displacement analysis measured motion in the direction of the gradient: The former analyzed the proportion of the total amount of cellular migration directed toward or away from the stimulus; the latter measured actual displacement toward or away from the stimulus over a fixed time period. Hence, the McCutcheon index measured chemotaxis only and was independent of velocity, whereas the directed linear displacement analysis measured velocity in addition to orientation of motion (see below). Thus, the displacement analysis more accurately measured the overall effect of the chemoattractant on the cell. Furthermore, analysis for discrete populations of cells differing in their chemotactic responses could be easily undertaken by this analysis. Because it was clear that the effect of stimulation did not yield a normal distribution of response, nonparametric statistical methods were applied to the data generated using the McCutcheon index and linear displacement analyses.

Measurement of angular orientation of motion accurately reflected the effect of the chemical gradient on the cell and is readily seen in the histograms illustrated. However, mathematical analysis was difficult. Zigmond analyzed orientation by measuring the percentage of cells oriented within defined limits toward the stimulus. This was useful but difficult to analyze statistically.

The Random Walk analysis was not useful in measuring chemotaxis in this short-term experimental system, as previously observed by Peterson and Noble.\textsuperscript{18} because of the tendency of cells to travel in an ortholinear pattern even in the absence of stimulus. Furthermore, there was no term in the Random Walk equation to give weight to movement in a given direction, as was the case with the McCutcheon index. Thus, the results obtained for stimulated cells were no different than those for unstimulated cells. Gail and Boone\textsuperscript{20} have used equations measuring "persistent" Random Walk to determine whether chemotaxis was present in slow-moving fibroblasts, which also tended to move without turning, whether stimulated or not. These equations could not be applied to the present data, as the observation time was insufficiently long; if the experiments were sufficiently prolonged, the gradients would no longer have been stable and the cells could have been in poor condition. Also, the persistent Random Walk analysis requires that any given cell travel at a constant velocity, a stipulation that was not met by the cell populations in the foregoing experiments.

Indirect evidence from refined analysis of data using the Boyden chamber suggested that chemokinesis (increased velocity without regard to directionality) was induced by many chemotactic agents. In the current experiments, a true increase in linear velocity could be separated from an apparent increase in velocity due to enhanced directionality. Linear velocity was indeed increased in the presence of the chemoattractant. This was not an artifact of more ortholinear motion of the cells undergoing chemotaxis, as diminution of the time interval over which translocation was measured did not alter the relative increase in calculated velocity of the stimulated cells as compared to the unstimulated cells. Also the frequency of turning was unchanged by the chemotactic gradient (Fig 5).

Attempts to measure changes of velocity in the presence of the chemooattractant but in the absence of a gradient between the two walls of the chamber were frustrated by the "edge effect," which was first noted by Zigmond.\textsuperscript{8} Under such conditions, the cells migrated toward the nearest well at increased speed. Orientation toward the nearest edge was seen either in unstimulated controls or in gradient conditions, regardless of the position on the bridge where the cells were observed. Zigmond has proposed that the "edge effect" is due to the utilization of the peptide by the cells at the center of the bridge, thus reducing the concentration and creating a gradient with both wells. The peptides have been shown to be degraded by membrane and soluble proteases in a rapid, receptor-independent process,\textsuperscript{13,14,21} and by slow intracellular processes following receptor-dependent internalization.\textsuperscript{14,16}

The present studies demonstrate the practicality of a visual method of analysis of chemotaxis and chemokinesis that is amenable to mathematical and statistical analysis. Using the techniques described, the contribu-
tions of chemotaxis and chemokinesis in the total biologic response of the cell to substances that alter motion can be separated. Because this technique relies on visualization of cells, the reactions of morphologically identifiable cells that may be present in mixtures of cells (eg, eosinophils, immature myeloid cells) may be readily analyzed without purification. Also, because analysis is carried out in the fluid phase, the reactions of cells that cannot pass the filter barrier of the Boyden chamber because of nuclear size, cytoplasmic granules, or membrane abnormalities can still be analyzed. The time of incubation is short, so the effect of unstable compounds can be more readily analyzed than in the Boyden chamber, in which incubations of 60–90 minutes are usually required. The incorporation of computer technology is of great utility in reducing the time necessary for analysis; however, the analyses described in this study can also be carried out without this aid. For these reasons, this system will facilitate visual analysis of cell motion and the influences upon it and will be very useful in understanding the mediators and modulators of cellular motility.

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The response of human neutrophils to a chemotactic tripeptide (N-formylmethionyl-leucyl-phenylalanine) studied by microcinematography

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