Chemokinetic Accumulation of Human Neutrophils on Immune Complex-coated Substrata: Analysis at a Boundary

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ABSTRACT The locomotory behavior of human blood neutrophil leukocytes was studied at a boundary between two surfaces with different chemokinetic properties. This was achieved by time-lapse cinematography of neutrophils moving on coverslips coated with BSA, then part-coated with immune complexes by adding anti-BSA IgG with a straight-line boundary between the BSA and the immune complexes. Cell locomotion was filmed in microscopic fields bisected by the boundary, and kinetic behavior was assessed by comparing speed (orthokinesis), turning behavior (klinokinesis), and the rate of diffusion of the cells on each side of the boundary, using a recently described mathematical analysis of kinesis. In the absence of serum or complement, the proportion of motile cells and their speed and rate of diffusion were greater on BSA than on anti-BSA, but there was no consistent difference in turning behavior between cells on the two surfaces. The immune complexes were therefore negatively chemokinetic in comparison with BSA, and this resulted from a negative orthokinesis with little or no contribution from klinokinesis. As would be predicted theoretically, this resulted in gradual accumulation of cells on the immune complexes even in the absence of a chemotactic factor. In further studies, a parallel plate flow chamber was used to show that, under conditions of flow, neutrophils accumulated much more rapidly on a surface coated with BSA-anti-BSA than on BSA alone. Moreover, neutrophils on immune complex-coated surfaces lost their ability to form rosettes with IgG-coated erythrocytes. This suggests that neutrophils on immune complex-coated surfaces redistribute their Fc receptors (Rfγ) to the under surface, and that the lowered speed of locomotion is due to tethering of neutrophils by substratum-bound IgG-Fc.

Of the behavioral mechanisms for leukocyte accumulation, chemotaxis is the best studied. Chemokinesis will result in cell accumulation under conditions where the kinetic factor is non-uniformly disposed (1). For example, if a population of cells is initially randomly distributed in a field in which one half has a higher concentration of a positive kinetic factor than the other, the cells will have a higher motility in the region of higher factor concentration. If the cells are otherwise unaffected by crossing the boundary between the two halves, it follows that each cell will generally spend less time in the high motility half than in the low motility half resulting in a gradual accumulation of cells in the low motility half. Cells will therefore initially cross the boundary from high to low motility more frequently than crossing it in the reverse direction until an equilibrium distribution is achieved in which the local density of cells is inversely related to their motility.

This predicted accumulation of cells assumes that there are no additional responses of the cells to the boundary dividing the field. For example, it is possible that some sort of chemotactic response to the boundary may totally prevent cells from crossing it in one direction. In this paper we test the prediction in a particular experimental system: the movement of leukocytes in a field with surface-bound antigen on one side and surface-bound immune complexes on the other.
antigen was BSA bound to a glass coverslip and a boundary was formed by adding anti-BSA IgG to one side to form surface-bound immune complexes. This system seems an ideal one to test, not only because of its cell biological interest, but also because immune complexes bind to physiological surfaces, such as vascular endothelia (2) and the glomerular basement membrane of the kidney (3, 4). Neutrophils bind to such adherent complexes and cause tissue injury by immune complex–mediated excytosis of hydrolytic enzymes (5, 6). Neutrophil accumulation in the renal glomeruli may be complement dependent (7) and therefore involve a chemotactic mechanism, but there are also models of glomerular damage in which neutrophil accumulation is complement independent (8, 9). Thus other mechanisms for accumulation than chemotaxis are worth studying. We also include here a report of the effect of surface-bound immune complexes on the adhesion of neutrophil leukocytes in vitro under conditions of flow, as a model for cell flow through the capillary bed in vivo. These measurements were made using a chamber in which neutrophils were allowed to flow across a surface coated with BSA or with BSA-anti-BSA at rates comparable to those occurring in the microvasculature (10). These studies, once again, test the hypothesis that leucocyte accumulation can occur in the absence of complement or of any source of a chemotactic gradient.

There have been few detailed analyses of cell locomotory behavior at boundaries between regions differing in their chemokinetic properties. One of the difficulties lies in obtaining unbiased estimates of such parameters of cell motility as speed, rate of turning, and rate of diffusion. The most common method of analyzing time-lapse films is to determine the position of the cell center at fixed intervals of a few frames, then to join these points so that the cell path is represented as a series of dots joined by straight lines. However, conventional methods of measuring speed and rate of turning directly from these segmented tracks suffer from the following bias: the values so obtained are determined partly by the length of the sampling time interval as well as by the cell behavior. We have therefore, in this paper, used a recently described method of analysis (11) in which unbiased estimates of parameters relating to speed and turning behavior are obtained by fitting a mathematical model of cell dispersion to the experimental data. One of the parameters of the model may be described as the root mean square (rms) 1 speed of the cells and the other as the persistence in direction time which is a reciprocal measure of the rate of change in direction with time of the cells. Since the rate of diffusion of cells is determined by their speed and their turning behavior, these two parameters suffice to describe all three aspects of behavior.2

This method of assessing speed and turning behavior allowed us to analyze kinesis behavior objectively. The rate of diffusion of cells may be thought of as a general measure of their motility which determines how many cells accumulate in different parts of their environment. Comparing two rates of diffusion, therefore, gives a general indication of kinesis. In contrast, the speed and persistence of cells may be thought of as particular aspects of motility, and comparing two speeds or two persisted times gives an indication of either the orthokinetic or klinokinetic component of kinesis, respectively. In many experimental systems, a portion of the cells remains stationary during a part or the whole of the experiment. These may be regarded simply as cells with zero speed which, when incorporated into the analysis, contribute to the estimate of orthokinesis, but in this paper, we have treated totally immobile cells separately since cell mobilization may have a different biological mechanism from speed changes or temporary stopping.

MATERIALS AND METHODS

Reagents: BSA (Miles Laboratories, Slough, England) was chosen as the antigen for study because albumin-coated surfaces are well known to support neutrophil locomotion. The rabbit anti-BSA was a DEAE-cellulose-purified IgG preparation donated by Dr. J. Michl (Rockefeller University, NY) and previously described by Michl et al. (12). This IgG antibody preparation and a control rabbit IgG preparation containing no anti-BSA activity were centrifuged at 100,000 g to remove aggregates before use to coat glass surfaces. Sheep erythrocytes were from Flow Laboratories (Irvine, Scotland). Rabbit anti-sheep erythrocyte antibody was a gift from Dr. D. A. R. Simmons (Bacteriology and Immunology Department, University of Glasgow). Hanks’ solution was from Flow Laboratories and was buffered with HEPES (10 mM) at pH 7.2.

Cells: Human neutrophils were separated from venous blood samples by the standard procedure using Dextran followed by Ficol-Hypaque. Contaminating erythrocytes were lysed with hypotonic Hanks’ solution (one part to four parts water). This procedure gave a cell preparation containing >95% neutrophils which were >98% viable.

Method for Studying Neutrophil Locomotion on Immune Complex–coated Surfaces: Detergent-cleaned coverslips (22 x 32 mm) were soaked in a solution of BSA (10 mg·ml–1) for 10 min, then washed thoroughly with water and air-dried. Preliminary experiments showed that this treatment gave a surface coat that allowed good locomotion of neutrophils in the absence of any fluid-phase protein. Only a small proportion of the protein became bound to glass (see Results). When the coverslips were dry, a thin streak of an anti-BSA IgG solution at between 1.5 and 3 mg·ml–1 was added in the way. One edge of a protein-free coverslip was moistened with a thin film of the antibody solution. This coverslip edge was touched lightly and rapidly onto the BSA-coated coverslip so that a thin strip of antibody solution was transferred to the latter as shown in Fig. 1. After 5 min, we washed the latter coverslip with Hanks-HEPES solution to remove excess antibody, making sure that the antibody was washed away in one direction only. We marked the position of the antibody streak by lightly scratching the coverslip with a diamond. The coverslip was then attached with silicone grease to a stainless-steel filming chamber whose dimensions were described earlier (13) so that the glass coated with BSA and with BSA-anti-BSA formed the lower surface of the circular well in the chamber. The well was filled with 0.35 ml of a suspension of neutrophils at 1 x 106·ml–1 in protein-free Hanks-HEPES solution, and sealed with a second, upper, coverslip to form a fluid-filled, air-tight compartment free of air bubbles. The chamber was placed on a microscope stage warmed to 37°C.

Neutrophils that settled onto the surface-bound BSA could be distinguished from those on immune complexes by Fc rosetting studies as described below. Also, cells landing on the complexes flattened out, whereas those landing on BSA alone remained round and refractile. The boundary between the BSA and BSA-anti-BSA complexes was located and a field was chosen for filming with one side containing cells moving on BSA-coated glass, the other side, cells moving on glass coated with BSA-anti-BSA. The fields chosen were on the left-hand side of the field as represented in Fig. 1, to exclude the possibility that antibody had been washed across the BSA-coated glass. We began filming ~5 min after warming the cells to 37°C, using a time-lapse interval of 4 s (15 frames per minute). Short segments of film (~20 min) were taken during this time, cells on both sides of the boundary retained viability judged by normal morphology and continued ruffling, even of immobilized cells. In control experiments, the above procedure was carried out using glass coated with BSA followed by non-anti-BSA rabbit IgG. Further experiments were
carried out in which the neutrophils were allowed to attach in the presence of 20% fresh normal human serum. This serum contained complement and should be activated by immune complexes; thus the complexes should act as a chemotactic gradient source.

**Method for Analysis of Locomotion:** The developed films were projected onto paper using an analytical projector (Lafayette Instrument Co., Lafayette, IN). Approximately 60 cells were chosen for analysis from the first frame of each experimental sequence. These cells were selected at random without knowledge of their subsequent behavior. The number of selected cells that had initially settled on each side of the boundary and the number of these that remained stationary throughout the sequence were recorded. For the motile cells, the cell center was marked at 10-frame intervals (40 s real time) and the dots were linked to form a track for each cell. If a cell had not moved by more than one cell radius during an interval, it was judged to be stationary. Stationary periods were included in the analysis of tracks of motile cells, but cells that remained immobile throughout the period of filming were treated separately. If a cell crossed the boundary during the experimental sequence, the portion of cell track on each side of the boundary was attributed to an appropriate data set so that the entire data from each film fell into two sets, one for each side of the boundary.

Further handling of the tracks was simplified by digitizing the successive cell positions (14). The square of cell displacement was calculated for single steps (40-s intervals) and for overlapping multiple steps (80-, 120-, 160-, and 200-s intervals). Thus the square displacement of the first double step of a track is given by \((x_2 - x_1)^2 + (y_2 - y_1)^2\); and of the second double step, \((x_4 - x_3)^2 + (y_4 - y_3)^2\). In general, the \(i\)th square displacement over \(j\)-tuple steps is given by \((x_{i+j} - x_i)^2 + (y_{i+j} - y_i)^2\). The data from steps that overlapped a stationary period were included in the following analysis, but those from steps that crossed the boundary and any subsequent steps in such cell tracks were ignored.

Next, for all the cell tracks and portions of tracks in one set, a value of rms displacement \(m_i\) was estimated. This is roughly the surface area covered by a single cell.

**Binding of \(^{125i}\)-BSA and \(^{125i}\)-IgG to Glass:** The amount of antigen and antibody bound to glass coverslips was calculated using \(^{125i}\)-BSA or \(^{125i}\)-anti-BSA IgG. (a) Coverslips were coated with \(^{125i}\)-BSA without antibody, to measure the amount of antigen bound. (b) Coverslips were coated with unlabeled BSA followed by \(^{125i}\)-IgG-anti-BSA, to measure the amount of antibody bound. (c) To correct for nonspecific binding of IgG, coverslips were coated with unlabeled ovalbumin followed by \(^{125i}\)-IgG-anti-BSA. Since the antibody in use did not cross-react detectably with ovalbumin, the amount of antibody bound was calculated by subtracting nonspecific IgG binding from total IgG binding, i.e., by subtracting \(c\) from \(b\). Protein binding was calculated per square millimeter, and from this the number of molecules bound per 100 \(\mu\)m \(^2\) was estimated. This is roughly the surface area covered by a single neutrophil.

**Fc Rosetting:** Sheep erythrocytes were coated with IgG by incubation with a subaglutinating dose of anti-sheep erythrocyte antibody in Hanks'-HEPES at 37°C for 60 min and then washed three times. Neutrophils at 10⁶ cells:mL⁻¹ were added to coverslips that had been coated with BSA followed by anti-BSA IgG or by control IgG, and were allowed to adhere at room temperature for 5 min. The coverslips were then washed in Hanks-HEPES and placed on ice. Antibody-coated sheep cells (0.05 uI at 1%) were added and the coverslips were incubated at 0°C for 1 h. Unattached erythrocytes were washed off by 15 dips in Hanks'-HEPES solution. The preparations were fixed with 1.25% glutaraldehyde, washed, and mounted, and counts were made of the percentage of rosetted neutrophils, defining a rosette as the presence of three or more attached erythrocytes per neutrophil.

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**Figure 1** Schematic drawing of the coverslip preparation for assaying neutrophil locomotion at a boundary. After coating the coverslip with BSA and then with IgG, we washed the coverslip in a left-to-right direction to avoid washing immunoglobulin across the boundary to be filmed.
**Table 1**

Percent Neutrophils Showing Fc-Rosettes

| Surface              | Condition A* | Condition B* |
|----------------------|--------------|--------------|
| BSA                  | 78.0         | 47.7         |
| BSA-antiBSA IgG      | 6.3          | 1.0          |
| BSA-control IgG      | 72.0         | NT           |

*NT, not tested.
* Cells adhered to surface for 5 min at 20°C and then rosetted for 60 min at 0°C.
* Cells adhered to surface for 60 min at 37°C and then rosetted for 60 min at 0°C.

Neutrophil Adhesion to Protein-coated Glass under Conditions of Flow:

Neutrophil adhesion to protein-coated glass was measured using a flow chamber of similar design to that of Doroszewski et al. (18) through which neutrophils could be perfused and their behavior recorded on videotape. A more detailed description of the system and its capabilities is the subject of a further report (22).

Experiments were performed under the same conditions throughout, i.e., at a fluid flow-rate of 1.2 mm/s within the chamber. Neutrophil suspensions (1 x 10^6 cells ml^-1) were kept on ice and considerable attention was paid to ensuring a monodisperse population by passing them through a 10-μm mesh Nitex filter (Plastok Associates, Birkenhead, United Kingdom, before use. The microscope slides were precoated with BSA (10 mg ml^-1) by filling the chambers with the protein solution for 30 min at room temperature. The BSA was then washed out of the chamber with Hanks'-HEPES solution and the chamber refilled with anti-BSA IgG, control IgG, or buffer solution for a further 20 min. The chambers were then washed with × 10 vol Hanks'-HEPES and used immediately.

The results are expressed as the number of adherent cells per unit area with time. In addition, the collection efficiency of the neutrophil suspension has been estimated at different times, i.e., the ratio of the number of adherent cells per unit area to the total number of cells presented to that area over a given period. This ratio has been calculated from the known parameters of flow rate, total cell number, and the dimensions of the chamber.

**RESULTS**

**Conditions for Neutrophil Locomotion**

**Antigen and Antibody Binding to Glass:** The quantity of BSA bound to glass was 2.8 ± 0.29 ng/mm² (mean ± SEM, four replicates) or 2.8 x 10⁶ molecules per 100 μm². The quantity of anti-BSA IgG bound to BSA-coated glass was 5.5 ± 0.19 ng/mm² (mean ± SEM, four replicates) or 2.2 x 10⁶ molecules per 100 μm² (after subtraction of nonspecifically bound IgG). Thus the ratio of antibody to antigen in the complexes on the glass was 0.8. The number of RFcγ on human neutrophils is 1.1-5 x 10⁷ per cell (19, 20, 23). Neutrophils seeded onto this immune complex-coated glass were likely to encounter an excess of IgG molecules. Whether this would saturate their Fc receptors would depend on surface IgG-Fc availability, recruitment of internalized RFcγ, and other factors.

**Fc Rosetting:** Cells attached to immune complex-coated surfaces redistribute their Fc receptors to the undersurface of the cell where the receptors bind to the substratum-bound IgG. Such cells lose the ability to show Fc rosettes. This has been shown for mouse macrophages (12), and in an unpublished study (P. C. Wilkinson and J. Michl) using human neutrophils, a similar loss of Fc rosetting on immune complex–coated glass was observed. Thus it seemed that a good way to confirm the position and sharpness of the boundary between the antigen-coated and the immune complex–coated glass would be to use Fc rosetting.

Table I gives the proportion of cells showing Fc rosettes on BSA and on BSA-anti-BSA. Most cells on BSA and very few cells on BSA-anti-BSA formed Fc rosettes at 0°C. If the cells were warmed to 37°C and allowed to move for 1 h, the same difference between cells on antigen and those on immune complexes was seen, though in this case, the proportion of rosette-forming cells on both surfaces was lower. Fig. 2 shows the appearance of the cells at the boundary between BSA and BSA-anti-BSA after Fc-rosetting. These cells had been kept on ice so that no movement had taken place. Cells on the immune complexes were Fc negative and showed spread morphology. Cells on BSA alone were rounded and most of them had formed Fc rosettes. The distinction between the two sides was sharp and allowed easy definition of the boundary.
Analysis of Cell Locomotion at the Boundary between BSA-coated and Immune Complex-coated Glass

Four film sequences of neutrophil locomotion at the boundary between BSA-coated and BSA-anti-BSA–coated glass in the absence of serum were analyzed. The data for individual cells from one of these sequences are shown in Table II. From the first frame of this sequence, 25 cells were chosen at random from the BSA side of the boundary for analysis, and 33 from the immune-complex side of the boundary. The film was then run on frame-by-frame and the cells were tracked for 150 frames (10 min real time). Of the 25 cells on BSA, 22 (88%) showed displacement and three remained stationary. Of the 33 cells on immune complexes, 17 (52%) showed displacement and 16 remained stationary. Thus more cells were immobilized on immune complexes than on BSA alone. Of the cells that moved, all tracks were analyzed except three of the tracks of cells on BSA and one on BSA-antiBSA. These cells moved out of the field of filming during the 10-min sequence and were therefore ignored. For the rest of the cells (19 on BSA; 16 on complexes), the speed ($S$) and the directional persistence time ($P$) were calculated (Table II). It is clear from Table II that neutrophils moved more rapidly on BSA than on immune complexes, and that the difference was significant. The persistence time of cells moving on immune complexes was shorter than that of cells moving on BSA. However, in three other experiments (Table III) no consistent difference in persistence time between the two surfaces was observed. The displacements of the two populations were

### Table II

**Speed ($S$) and Persistence Times ($P$) of Individual Neutrophils, from a Single Film Sequence, Moving on Different Surface Coats**

| Track no. | $S$ (μm/min) | $P$ (min) |
|-----------|--------------|-----------|
| 1         | 21.9         | 0.65      |
| 2         | 15.4         | 0.35      |
| 3         | 15.2         | 0.88      |
| 4         | 16.8         | 0.6       |
| 5         | 14.9         | 1.4       |
| 6         | 11.5         | 0.64      |
| 7         | 12.8         | 1.14      |
| 8         | 16.8         | 0.43      |
| 9         | 22.6         | 1.03      |
| 10        | 15.4         | 0.77      |
| 11        | 24.9         | 0.46      |
| 12        | 12.9         | 1.06      |
| 13        | 25.8         | 0.63      |
| 14        | 14.6         | 1.75      |
| 15        | 13.9         | 0.71      |
| 16        | 19.8         | 0.7       |
| 17        | 17.9         | 0.87      |
| 18        | 20.4         | 1.17      |
| 19        | 14.6         | 1.08      |

rms* ± SEM 16.34 ± 0.81 0.75 ± 0.09 8.04 ± 0.36 0.61 ± 0.09

* rms $S$ and $R$ are derived from line-fitting on total data set, not from simple mean ± SEM for individual cells.

### Table III

**Analysis of Locomotor Behavior of Neutrophils in Four Experiments at a Boundary between BSA and Immune Complexes, and in a Control Experiment at a Boundary between BSA and BSA + Control IgG**

| Exp. | Surface | Total cells | No. of motile cells | $S$ (μm/min ± SEM) | $P$ (min ± SEM) | $R$ (μm²/min ± SEM) |
|------|---------|-------------|---------------------|---------------------|----------------|-------------------|
| I    | Control: BSA/BSA + control IgG | BSA | 28 | 0.64 | 18 | 16 | 8.64 ± 0.32 | 0.85 ± 0.12 | 127 ± 21 |
|      | BSA-antiBSA | BSA + IgG | 29 | 0.59 | 17 | 19.8 | 9.09 ± 0.41 | 0.84 ± 0.14 | 139 ± 30 |
| II   | BSA | 25 | 0.88 | 19 | 3.6 | 16.34 ± 0.81 | 0.75 ± 0.09 | 400 ± 56 |
|      | BSA-antiBSA | 33 | 0.52 | 16 | 19.6 | 8.04 ± 0.36 | 0.61 ± 0.09 | 79 ± 10 |
|      | BSA | 28 | 0.96 | 20 | 10.0 | 17.90 ± 0.77 | 0.77 ± 0.1 | 493 ± 59 |
|      | BSA-antiBSA | 31 | 0.8 | 20 | 21.8 | 10.90 ± 0.55 | 0.89 ± 0.14 | 211 ± 44 |
| III  | BSA | 28 | 0.96 | 23 | 11.9 | 15.49 ± 0.72 | 0.76 ± 0.12 | 365 ± 55 |
|      | BSA-antiBSA | 29 | 0.68 | 17 | 25.1 | 10.71 ± 0.58 | 0.77 ± 0.15 | 177 ± 30 |
|      | BSA | 29 | 0.96 | 25 | 15.3 | 11.83 ± 0.5 | 0.68 ± 0.1 | 190 ± 23 |
|      | BSA-antiBSA | 36 | 0.8 | 17 | 42.8 | 6.38 ± 0.32 | 1.12 ± 0.17 | 91 ± 12 |
measured and did not deviate significantly from random. Thus there was no evidence for chemotaxis of either population.

Table II includes only the tracks of cells that remained on the side of the boundary they started on. If a cell moved across the boundary, only the portion of track on the starting side was included in this table. Crossing behavior was considered separately. In this group of cells, five crossed from BSA to immune complexes and two from complexes to BSA. The speed of the five cells crossing from BSA to complexes was 15.26 ± 2.7 μm·min⁻¹ on BSA but 9.5 ± 1.3 μm·min⁻¹ on the complexes. The speed of the two cells crossing from complexes to BSA was 8.8 ± 1.8 μm·min⁻¹ on the complexes and 17.0 ± 1.7 μm·min⁻¹ on the BSA. These results suggest that cells change speed when crossing the boundary, although the sample size is very small. Further experiments of this sort are discussed below.

Table III is a summary of the locomotory behavior of neutrophils in four experiments where locomotion was filmed in relation to a boundary between BSA and immune complexes without serum and in one experiment where locomotion on BSA was compared with locomotion on BSA plus control IgG. Experiment I is the experiment detailed in Table II. It should be noted that these experiments were done on different days using cells from different individuals. Therefore speeds and other parameters cannot be compared between the different experiments, though the comparison of cells on BSA and on immune complexes within each single experiment is valid. In all experiments, the cells moved faster on a BSA-coated surface than on a surface coated with immune complexes. Also the moving cells spent a higher proportion of the time in motion on BSA than on immune complexes. Furthermore, there were fewer immobilized cells on the BSA-coated surface than on the complexes. However, there was little difference in P between the two surfaces, suggesting that turning behavior was similar on both. In none of the experiments was there any significant net displacement of the cell population; thus there was no evidence for chemotaxis. In two control experiments using BSA and control IgG (one shown in Table III), no difference in the parameters S, P, R, or M was observed between the two sides of the boundary. Because of the variation in these parameters between different cell batches evident in Table III, controls and experimental groups can be compared most easily by comparing the kinetic indices, and these are shown in Table IV. Most of the negative chemokinetic effect of immune complexes can be seen to be due to negative orthokinetic. These experiments suggest that immune complexes decrease the rate of diffusion of neutrophils, partly by immobilizing a portion of the cells, partly by reducing the speed of the remainder.

### Table IV

| Kinetic Indices for the Experiments Presented in Table III |
|----------------------------------------------------------|
| **Mobilization index** | **Orthokinetic index** | **Klinokinetic index** | **General kinetic index** |
|------------------------|------------------------|------------------------|--------------------------|
| log₂ m' / m       | log₂ s' / s     | log₂ p' / p  | log₂ r' / p = KL + SOI |
| Control               | -0.116               | +0.073                  | -0.017                  | +0.130                  |
| Exp. I                | -0.757               | -1.020                  | -0.296                  | -2.339                  |
| Exp. II               | -0.262               | -0.714                  | +0.206                  | -1.226                  |
| Exp. III              | -0.498               | -0.532                  | +0.202                  | -1.043                  |
| Exp. IV               | -0.262               | -0.890                  | +0.717                  | -1.063                  |

The number of cells crossing the boundary between BSA and immune complexes in the four experiments shown in Tables III and IV is similar to the rate of blood flow in small venules, neutrophils contacted and adhered to BSA-coated glass, and the number of adherent cells increased linearly with time (Fig. 3). The percentage of cells adhering to the glass (collection efficiency) gradually increased with time but was not >30% (Table VI). Under identical conditions the adhesion of neutrophils to BSA-anti-BSA-coated glass was much greater (Fig. 3) with a collection efficiency after 5 min of 90.4% (Table VI). In addition, the percentage of spread cells (i.e., totally

### Table V

**Speed of Cells Crossing from BSA- to Immune Complex-coated Glass and Vice Versa (μm/min ± SEM)**

| Surface | Cell crossing |
|---------|--------------|
| BSA     | 12.72 ± 0.9  |
| BSA-antiBSA | 10.25 ± 0.6 |

*44 cells. Paired sample Student's t test: t = 2.7; df = 43; P < 0.005.

**Crossing Behavior at the Boundary and Accumulation on Immune Complexes**

The number of cells crossing the boundary between BSA and immune complexes in the four experiments shown in Tables III and IV is similar to the rate of blood flow in small venules, neutrophils contacted and adhered to BSA-coated glass, and the number of adherent cells increased linearly with time (Fig. 3). The percentage of cells adhering to the glass (collection efficiency) gradually increased with time but was not >30% (Table VI). Under identical conditions the adhesion of neutrophils to BSA-anti-BSA-coated glass was much greater (Fig. 3) with a collection efficiency after 5 min of 90.4% (Table VI). In addition, the percentage of spread cells (i.e., totally

### Effect of Adding Fresh Serum

When neutrophil locomotion was assayed at a boundary as described above, but with the addition of fresh human serum (20%) to the fluid phase, chemotaxis of cells on the BSA towards the immune complexes was seen. For 13 cells whose starting position was on BSA within 100 μm of the boundary, the mean velocity of locomotion towards the complexes was 2.7 μm·min⁻¹ ± 0.4 SEM (P < .001). Velocity in the axis parallel to the boundary was 0.3 μm·min⁻¹ ± 0.5 (not significant).

### Neutrophil Adhesion to BSA-coated and Immune Complex-coated Glass under Conditions of Flow

The adhesion of neutrophils to BSA and to BSA-anti-BSA-coated glass was tested in the flow chamber system described in Materials and Methods. At a flow rate of 1.2 mm·s⁻¹, which is similar to the rate of blood flow in small venules, neutrophils contacted and adhered to BSA-coated glass, and the number of adherent cells increased linearly with time (Fig. 3). The percentage of cells adhering to the glass (collection efficiency) gradually increased with time but was not >30% (Table VI). Under identical conditions the adhesion of neutrophils to BSA-anti-BSA-coated glass was much greater (Fig. 3) with a collection efficiency after 5 min of 90.4% (Table VI). In addition, the percentage of spread cells (i.e., totally
and one of our major objectives here was to test a method of calculating the true speed and turning behavior of cells which has been presented as a theoretical model (11) but not used experimentally before, and that should allow comparisons to be made between the kinetic parameters derived from different experiments or from different laboratories. Our experiments suggested that neutrophils on immune complex–coated surfaces moved more slowly than on BSA and that a greater proportion of the cells were immobilized on immune complexes than on BSA, but that there were not consistent differences in turning behavior between the two sides. This decrease in speed led to a decrease in the calculated diffusion rate of the cells on immune complexes in comparison to that on BSA. As predicted, this resulted in a gradual accumulation of neutrophils on the immune complexes.

The considerable variation in locomotor behavior between different neutrophil populations prepared on different days is worthy of comment. The reason for this variation is not known precisely, but it may reflect differences in preparative procedures. For example, unpublished observations (Shields, J. M., and W. Haston) suggest that if neutrophils are not cooled below room temperature during preparation, or if an erythrocyte-lysis step is avoided, a higher proportion of the cells remains rounded when they are seeded onto BSA-coated surfaces. Thus neutrophils may assume locomotor morphologies in response to stimuli introduced during preparation. In any case, the variation between individual batches of cells means that comparisons between different experiments can only be made using the “kinetic indices” (Table IV) and not for statistical purposes.

flattened) within the adherent population was much higher on the immune complex–coated glass (Table VI). In contrast, the number of adherent cells, the collection efficiency, and the percentage of spread cells was markedly less on control non-anti-BSA-IgG-BSA–coated glass, although these values were slightly greater than on BSA-coated glass alone (Fig. 3; Table VI). These data complement the cell locomotion data showing that neutrophils on immune complex–coated surfaces were more frequently immobilized and support the view that their immobilization was due to increased substratum adhesiveness.

DISCUSSION

The idea that neutrophil and other leukocytes show chemokinesis has gained wide acceptance since the term was reintroduced into the leukocyte literature (16). However, there has not been any experimental work analyzing the chemokinetic behavior of neutrophils as a determinant of cell accumulation. As pointed out by Dunn (1), estimates of speed and turning behavior of cells vary with the method used to measure them, and one of our major objectives here was to test a method of calculating the true speed and turning behavior of cells which

| Flow time (min) | BSA | AntiBSA-BSA | Control IgG-BSA |
|----------------|-----|------------|----------------|
|                | T   | S          | CE             |
| 1              | 4   | 0          | 15.3           |
| 2              | 43  | 11.6       | 20.6           |
| 5              | 92  | 11.9       | 27.7           |

*Flow time T S CE T S CE T S CE

T, total number of adherent cells. S, percentage of spread cells. CE, collection efficiency percent = No. of adherent cells/total no. of cells flowing through chamber x 100.

### TABLE VI

**Neutrophil Adhesion to Protein-coated Glass under Flow Conditions**

![Graph showing neutrophil adhesion to protein-coated glass under flow conditions](image-url)
binding to further IgG molecules until no free receptors remain available for binding to newly added IgG. These cells therefore fail to form Fc rosettes. Thus negative orthokinesis is due to cell tethering to the substratum by bound complexes. A similar mechanism seems to us to furnish a plausible explanation for the accumulation of neutrophils in vivo on the vascular basement membrane following immune complex deposition, for example in the glomeruli. It is unlikely that chemotactic gradients operate under high flow conditions within the glomerular capillaries, and we would suggest that what we have observed in the flow chamber is probably similar to what happens to leukocytes within these capillaries, namely adhesive trapping. The lack of a requirement for chemotaxis under flow conditions is supported by observations that C3-depletion does not prevent neutrophil deposition on basement membranes bearing deposited immune complexes (9).

The accumulation of neutrophils at extravascular sites of immune complex deposition probably takes place by several mechanisms, of which we have isolated a single one for study here. Where active locomotion of neutrophils from blood vessels is required, complement-generated chemotactic gradients would provide the best means of ensuring rapid neutrophil accumulation. If no such gradient were present, any leukocyte-type bearing Fc receptors would still accumulate on immune complexes by negative chemokinesis and adhesive trapping. Once bound to the complexes, neutrophils would secrete a variety of factors which might recruit further neutrophils by chemotaxis, either directly (e.g., released leukotriene B₄) or indirectly by activating complement, as has been shown for proteases released by cells. It may be that negative chemokinetic effects have their greatest importance in chronic inflammations, where there is a gradual accumulation of inflammatory cells, including Fc-positive lymphocytes and macrophages, and where a number of mechanisms other than chemotaxis are likely to be operative in the maintenance of the cellular lesion. It would certainly be interesting to study the chemokinetic behavior of macrophages or lymphocytes at immune-complex boundaries, though it would probably also prove more difficult technically than the study of neutrophils.

P. C. Wilkinson was supported by a grant from the Medical Research Council.

Received for publication 23 February 1984, and in revised form 12 July 1984.

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