remainder subjected to ultrasonics after its addition, until the appearance of the respective colloid-associated colours. In order to assess the particle size and exact dispersion of the colloids poly-L-lysine treated[7] pioloform-F coated grids were submerged in the respective suspensions (see legend) and examined electronmicroscopically.

Initial observations indicated that the average particle diameter of the ultrasonically induced colloid was less than 10 nm whereas that of the heat preparation was considerably larger at around 25 nm. Both preparations were highly disperse. To quantify this observation a number of ultrasonic and heat induced sols were randomly photographed and the particle diameters measured on a projection screen. Arithmetical means and standard deviations were calculated for each preparation. An analysis of the data showed that the mean diameters of the ultrasonically induced colloids differed significantly (t-test, $p < 0.05$) within a range of 6–10 nm with an occasional average of less than 5 nm. Heat produced colloids also varied significantly in diameter within a range of 20–30 nm. Furthermore the standard deviations within each group differed significantly (F-test applied to the variances, $p < 0.05$) and coefficients of variation of 8–50% were found in both groups.

The sols were still disperse after several weeks without stabilization[8]. The ease of preparation, however, allowed fresh batches to be made whenever required. The colloidal gold adsorbed $\gamma$-globulins and protein A[9] readily, as demonstrated with sodium chloride flocculation[10], and therefore it is suitable as an immunological marker. A similar colloidal gold was successfully prepared using a laboratory ultrasonic cleaner.

Although the mechanism with which the ultrasonic energy induces the colloid is unclear, further limited observations showed that variations in pH of 5.0–8.5 as well as in alcohol concentration of 0.5–10% appeared not to influence the results obtained, contrary to experience obtained with conventional methods.


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## Chemotaxis of human neutrophils against gravity: A new method[1]


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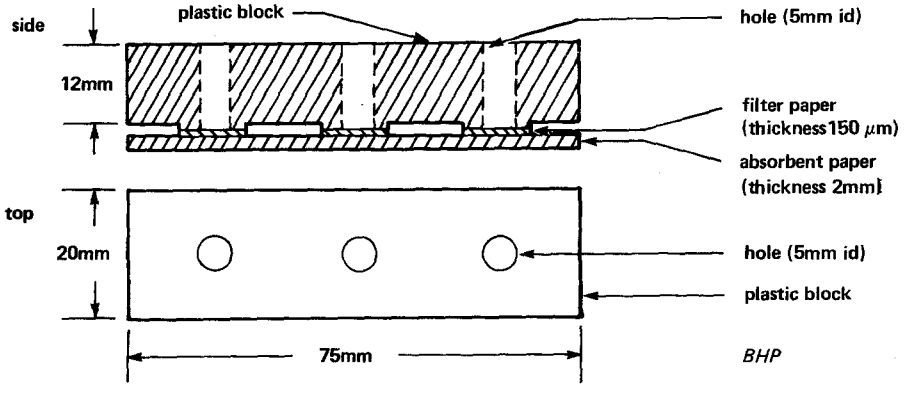
*Summary.* A new method is described in which human neutrophils were made to migrate upward and against gravity. Thus, the possible effect of gravity on cell migration and consequent detachment of cell after migration have been eliminated.


The Boyden Chamber method[2] is widely used for studies in chemotaxis, and is based on the assumption that the migrated cells would remain attached to the lower surface of the filter where they are counted. However, a variable proportion of migrated cells may detach from the lower surface[3]. Thus, the cell count at the lower surface of the filter may not always represent the actual number of cell migration. To overcome this inherent problem, I have devised a simple method in which the cells are made to migrate upward through the filter membrane, and thus the possibility of spontaneous detachment after migration is eliminated. 3 ml of fresh venous blood (heparin 20–40 units/ml) was centrifuged in a 5 ml plastic tube at 300 g for 10 min. The plasma, buffy coat and the upper layer of red blood cells were transferred into a new 5 ml plastic tube and mixed gently. The tube was placed upright in a test tube rack at room temperature and the red blood cells settled for 30–45 min. The leukocyte rich plasma layer was then transferred into a new plastic tube, the total cell as well as polymorphonuclear leukocytes (PMN) were counted by the




A diagram showing the apparatus used for leukocyte deposition.

standard method. The leukocyte suspension was then centrifuged at 150 × g for 10 min at room temperature, the plasma removed, and the leukocytes resuspended in Medium 199 to make a final concentration of $3 \times 10^6$ PMN/ml. The recovery of leukocytes ranged between 60 and 70%. The final leukocyte suspension consisted of PMNs (50–70%), and 10–20 red blood cells per leukocyte.

The key point of this method is to place (or sandwich) the leukocytes between the 2 filter papers. After a small drop (about 10 µl) of Medium 199 is placed on a thick absorbent paper, a pre-wetted Millipore filter paper (5 µm pore size) is placed on the absorbent paper so that the filter paper is centered on the spot where Medium 199 was placed. Then, a plastic cube (1 × 2 × 7 cm) with 3 separate holes (5 mm in diameter) is placed on the filter paper so that each hole is centered with respect to each filter paper (figure). Thus, a well with the filter at the bottom is created. While pressing the plastic cube downward with 1 hand, 50 µl of cell suspension is delivered twice to the bottom of the well ($3 \times 10^5$ PMNs). Within a few sec, the fluid of cell suspension is absorbed into the absorbent paper leaving cellular elements on the surface of the filter within the circular area of 5 mm in diameter. Quickly, the filter paper is removed from the plastic cube and the leukocytes are sandwiched by placing another wet filter paper on top of the leukocyte deposits. Care must be taken to avoid an air trap between the 2 filters. The 2 filter papers with sandwiched leukocytes are placed in a Boyden Chamber so that the filter with leukocyte deposits faces upward. After placing the screw tightly, Medium 199 is introduced into the lower compartment while the upper compartment is simultaneously filled with zymosan activated serum as a chemotactic attractant. Coverslips were placed onto the openings of the chambers so that the space between the coverslip and the chamber was sealed by capillary osmosis of overflowing fluid. The entire chamber was immersed into a water bath (37 °C) so that the upper surface of the chamber is just above the water level and incubated for 2 h or an appropriate time period.

After incubation, the fluid in both the compartments was removed with care not to disturb the migrated cells, and the 2 filters separated, fixed in 100% methyl alcohol for 2–3 sec and stained with Hematoxylin using the method of Boyden[2] with minor adaptation. Only those cells that had completely migrated through and reached the upper surface of the filter were counted using magnification × 400. The average number of PMNs per high power field is defined as a migration index (MI), which is shown in the table. The maximum number of PMNs (115 ± 20) was reached after 2 h of incubation. The spontaneous migration (or random movement) of PMNs was found to be minimal, ranging 0–1 PMNs/high power field. Some of the PMNs were found within the lower filter as far downward as 100 µm, but few completed migration down to the lower surface. The number of PMNs migrated to the top of the filter were directly proportional to the number of PMNs loaded initially (table).

I have used this method for the past 3 years, and have found it to be reliable and reproducible. It has been most useful in the study of neutrophil chemotaxis of some patients with diabetes mellitus, in which the spontaneous detachment of migrated neutrophils may frequently take place.

Effects of incubation time and number of cells loaded on neutrophil migration index of healthy volunteers (Mean ± ISD, N = 25)

| Incubation time | Migration index |
|---|---|
| 30 min | 42 ± 12 |
| 60 min | 95 ± 15 |
| 120 min | 115 ± 20 |
| Number of neutrophils loaded (120 min incubation) | |
| $1 \times 10^5$ | 28 ± 8 |
| $2 \times 10^5$ | 62 ± 12 |
| $3 \times 10^5$ | 115 ± 20 |

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## PRAEMIA

### Ruzicka-Prize 1980

Every year, a prize from the Ruzicka-Prize Fund is awarded to a young research worker for an outstanding work in the field of general chemistry that has already been published and achieved in Switzerland or by a Swiss national abroad. Proposals for candidates may be submitted before June 30th, 1980 at the latest to the President of the Board of the Swiss Federal Institutes of Technology, ETH-Zentrum, 8092 CH-Zürich.

## CURSUS

### Italy

### Ispra Courses 1980

The Ispra establishment of the joint research centre of the Commission of the European Communities presents its 1980 programme of courses and seminars. Brochure with detailed description of the courses is available at the Secretariat Ispra-Courses, Centro Comune di Ricerca, I-21020 Ispra/Varese (Italy).

## CONGRESSUS

### Italy

### 7th international symposium on mass spectrometry in biochemistry, medicine and environmental research

*Milan, 16–18 June 1980*

For information write to: Dr Alberto Frigerio, Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, 62, I-20157 Milano/Italy.

### Czechoslovakia

### International conference on xenobiochemistry

*Bratislava, 9–13 June 1980*

The conference is held on 'Biochemistry of metabolism and effect of xenobiotics'. Detailed information by: Prof. A. Jindra, Biochemical Institute, Kalinciekova 8, 88034 Bratislava/CSSR.