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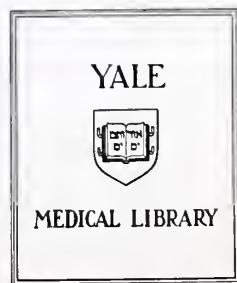
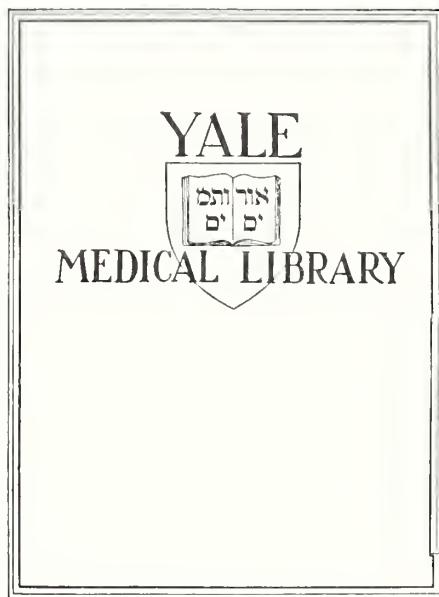
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LOW DOSE EFFECTS OF COLCHICINE
AND VINCRISTINE ON HUMAN
POLYMORPHONUCLEAR
LEUKOCYTE MIGRATION



ROBERT ALEX CAPLAN

1977



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LOW DOSE EFFECTS
OF COLCHICINE AND VINCRISTINE
ON HUMAN POLYMORPHONUCLEAR LEUKOCYTE MIGRATION
IN A MODIFIED AGAROSE PLATE SYSTEM

Robert Alex Caplan

B. A. Harvard University, 1973

A thesis submitted to
the faculty of the School of Medicine
Yale University
in partial fulfillment of the requirements for
the degree of Doctor of Medicine

New Haven, Connecticut

March 1977

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ABSTRACT

A modified agarose plate technique was used to investigate the effect of colchicine and vincristine on the migration of human polymorphonuclear (PMN) leukocytes. Agarose plates were prepared by the method of Clausen, except that colchicine and vincristine were added to the nutrient medium to achieve drug concentrations of 10^{-9} to 10^{-5} M. A hexaxial reference system was devised for time-course studies of cell migration.

Three drug-related effects were observed. Loss of sharpness at the peripheral edge of cell migration was seen at colchicine concentrations of 10^{-8} M and greater, and at vincristine concentrations of 10^{-7} M and greater. Change in central cell aggregation from a disperse to a dense pattern was found at colchicine and vincristine concentrations of 10^{-7} M and greater. Time-course studies revealed significant inhibition of radial cell migration at colchicine concentrations of 10^{-7} M and greater, and at vincristine concentrations of 10^{-8} M and greater.

Following a therapeutic intravenous dose of colchicine, there is now preliminary evidence that leukocytes sequester the drug at a concentration of approximately 10^{-7} M for at least 24 hours. In the present study, the demonstration of migration inhibition and changes in agarose plate migration pattern at a similar drug concentration may lend support to the hypothesis that the inflammatory action of colchicine in acute gouty arthritis is attributable in part to altered leukocyte mobility.

2.

The observations in this study also confirm an earlier report, in a different experimental system, of diminished leukocyte mobility in the presence of 10^{-7} M colchicine.

INTRODUCTION

Studies of inflammation first led to an appreciation of PMN leukocyte mobility. Using a crystal of salt as an irritant stimulus, Addison (1843) initiated studies of vascular changes accompanying inflammation in the transparent web of the frog's foot. He noted that leukocytes collected in the extravascular tissue after an initial period of accumulation along capillary walls. Shortly thereafter, his observations were extended by Waller (1848) who reported emigration of leukocytes from the intact capillary lumen into the extravascular space of inflamed areas of the frog's tongue. A series of similar studies by Cohnheim (1882) led to his classical description of leukocyte migration through the capillary wall:

A pointed projection is seen on the external contour of the vessel wall; it pushes itself further outwards, increases in thickness, and the pointed projection is transformed into a colourless rounded hump; this grows longer and thicker, throws out fresh points, and gradually withdraws itself from the vessel wall, with which it is connected only by a long thin pedicle. Finally this also detaches itself, and now there lies outside the vessel a colourless, faintly glittering contractile corpuscle...

The early observations of PMN leukocyte locomotion in amphibians were later extended to mammalian species. Leber (1888) demonstrated leukocyte accumulation in rabbit and guinea pig corneas which had been injected with extracts of S. aureus. In freshly excised sections of cornea, he reported that leuko-

cyte migration was oriented toward the initial site of injection, thus providing an early example of chemotaxis. Sanderson (1924) devised a transparent observation chamber which supported a thin layer of living tissue. The device was suitable for implantation in the tadpole tail or rabbit ear. Using a modified form of this chamber, Clark and coworkers (1936) studied inflammation in the rabbit, and confirmed the sequence of events originally described by Cohnheim. Chamber studies in the tadpole tail provided further evidence of PMN leukocyte chemotaxis.

Studies of leukocyte mobilization in the human inflammatory response were first made practicable by the introduction of the skin window technique (Rebuck and Crowley, 1955), in which cells appearing at the site of an experimental skin lesion were collected on a succession of glass coverslips for permanent fixation and staining. Although emigration of cells from blood vessels could not be observed directly, the orderly and classical progression of PMN leukocytes, lymphocytes, and monocytes was documented in repeated trials. Subsequently, this technique was utilized by Perillie and Finch (1960) and by Boggs (1960) to assess the inflammatory response in patients with acute leukemia. In this population, delayed and diminished PMN leukocyte mobilization at the skin window site was found, suggesting an experimental correlate for the increased susceptibility to bacterial infection which is observed in acute leukemia. A study of patients with poorly controlled diabetes mellitus and ketosis also revealed delayed and diminished mobi-

lization of PMN leukocytes (Perillie et al., 1962).

Better quantitation of leukocyte mobilization in man was achieved by Perillie and Finch (1964) through the design of a cup chamber which could be secured over the site of an experimental skin lesion and filled with fluid. The chamber was equipped with two outlets to permit sequential collection of fluid for total and differential cell counts. Initial studies with this chamber revealed a decrease in early PMN leukocyte mobilization as well as a decrease in total white cell mobilization in patients with acute leukemia. In a later study, Holland and coworkers (1971) reported a series of patients with acute myelogenous leukemia in which initially depressed values for PMN leukocyte mobilization and clearance returned to the normal range during remission. A retrospective report (Senn and Holland, 1967) suggested that a low value for PMN leukocyte mobilization in acute leukemia might serve as a useful indicator of subsequent risk of serious bacterial infection.

A number of techniques for the study of PMN leukocyte locomotion in vitro have also been described. Comandon (1917, 1919) suspended whole blood and attractant material between a glass slide and coverslip and then followed PMN leukocyte movement by time-lapse cinemicrography; chemotaxis toward parasitized erythrocytes and starch granules was observed. McCutcheon and coworkers (1934) made detailed camera lucida drawings of PMN leukocyte migration in slide and coverslip preparations. On the basis of path configurations and frequency

of cell contact with the test stimulus, they reported strong attraction of leukocytes towards S. albus and weak attraction towards autologous dead leukocytes. Harris (1953) refined the slide and coverslip technique by studying relatively pure preparations of PMN leukocytes. These preparations were obtained by preincubation of whole blood on glass slides, followed by gentle rinsing to remove non-adherent cells. Harris found that bacteria differed in their action on PMN leukocytes in vitro: S. albus and C. diphtheriae were chemotactic; S. aureus and S. pyogenes had toxic effects; and B. anthracis and B. friedländeri had no demonstrable action. Recently, investigators have used modifications of the slide and coverslip technique to study PMN leukocyte mobility in the presence of antimetabolic agents (Ramsey and Harris, 1972) and to analyze the inherent randomness of leukocyte locomotion in non-chemotactic environments (Peterson and Noble, 1972). The results of these later studies are considered in the Discussion section of this paper.

Ketchel and Favour (1955) developed a prototype system for the study of PMN leukocyte migration in capillary tubes. Using a microscope equipped with an ocular micrometer, they recorded the progressive migration of PMN leukocytes from the buffy coat formed by centrifugation of weakly heparinized blood. Their technique was later modified by the use of purified leukocyte suspensions in place of whole blood (Miller et al., 1971). In the contemporary literature, decreased leukocyte mobility in capillary tubes has been described in association with the lazy leukocyte syndrome (Miller et al., 1971) and membrane-

dialysis of leukocytes (Henderson et al., 1975). The findings of the study by Henderson's group are particularly striking in that capillary tube migration was completely abolished in leukocytes which had been passed through a cellulose acetate (Dow) membrane, while migration was only slightly diminished by passage through a polysulfone (Amicon) membrane.

A major advance in methodology occurred in 1962 with Boyden's design of a two-compartment system for quantitative measurement of PMN leukocyte chemotaxis. Boyden's original device consisted of two perspex chambers, vertically stacked and separated by a porous membrane filter. Leukocytes in suspension were injected into the uppermost chamber and a test substance was placed in the lower chamber. Chemotactic effect was assessed by comparing PMN leukocyte penetration of the membrane filter in the presence of test material with penetration in the presence of control solution. Many modifications of the original chamber have been described, the most important being the addition of a small-pore filter beneath the porous membrane filter to increase the efficiency of leukocyte collection (Keller et al., 1972). Boyden's technique has been applied to the study of PMN leukocyte chemotaxis in a variety of clinical disorders, with decreased function noted in the Chełciak-Higashi syndrome (Clark and Kimball, 1971), the lazy leukocyte syndrome (Miller et al., 1971), and a familial chemotactic defect (Miller et al., 1973). Defective chemotaxis has also been reported in patients with toxic granulation of neutrophils (McCall et al., 1971), alcoholic liver disease

(DeMeo and Andersen, 1972), juvenile diabetes (Miller and Baker, 1972; Hill et al., 1974), and adult-onset diabetes (Mowat and Baum, 1971).

The present paper introduces a new approach to the study of PMN leukocyte migration. The basic methodology is derived from an agarose plate technique which was first implemented by Carpenter and coworkers (1968) in studies of guinea pig tissue cell migration, and later extended by Clausen (1971) to studies of human PMN leukocyte migration. In Clausen's system, cells are applied to a central well in an agarose plate and allowed to migrate radially along the interface between the nutrient agarose gel and the surface of the supporting plastic petri dish. Presensitized lymphocytes (Gaines et al., 1972; Astor et al., 1973) or their soluble factors (Clausen, 1972) are mixed with the PMN leukocytes to detect inhibitory effects. The technique has been used primarily in the field of immunology as an indicator system for lymphocyte activation and lymphokine release.

In the present study, low concentrations of colchicine and vincristine are added to the nutrient agarose gel to evaluate the effects of these antimitotic agents on PMN leukocyte mobility. Lymphocytes are removed from the PMN leukocyte preparations at an early step to avoid extraneous inhibition. A special marking system is used to permit time-course studies of cell migration. The results obtained in this study are reproducible and compatible with other recent

work, suggesting that a modified agarose plate technique may have further utility in studies of PMN leukocyte locomotion.

METHODS

Study site

The work reported here was conducted in the Clinical Laboratory Division of the Radiation Effects Research Foundation in Hiroshima, Japan. The Foundation is a cooperative Japanese and American research organization, engaged in studies of the long-term, radiation-related effects of the atomic detonations in Hiroshima and Nagasaki.

Consent and authorization

For studies of PMN leukocyte migration, 10 ml blood specimens were obtained from healthy, previously informed, adult volunteers. None of these individuals had a history of exposure to the initial atomic detonation either in Hiroshima or Nagasaki. For pilot studies of PMN leukocyte isolation, 5 ml blood specimens were obtained from healthy and hematologically normal members of the Adult Health Study (AHS) population in Hiroshima. The AHS population is a carefully characterized set of atomic bomb survivors and matched controls, examined at two-year intervals. Authorization for use of small blood samples from this population was obtained by approval of a research protocol submitted to the Directors of the Radiation Effects Research Foundation in Hiroshima.

Sample collection

Venous blood was drawn from the antecubital fossa through a #20 or #21 disposable needle into a sterile plastic syringe. The usual sample volume was 5 or 10 ml. The blood was delivered

into a sterile glass tube, and thoroughly mixed with an equal volume of calcium- and magnesium-free Hank's Balanced Salt Solution (HBSS), pH 7.2, containing 10 IU/ml sodium heparin. Cell separation procedures were then initiated promptly.

Isolation of PMN leukocytes

Modification of the Boyum-Thorsby techniques. Boyum (1968) originally developed a rapid and efficient technique for isolation of granulocytes and lymphocytes from whole blood. The technique utilized dextran sedimentation of erythrocytes, followed by Ficoll-Hypaque flotation of the remaining white cell supernatant to separate lymphocytes and granulocytes. Thorsby (1967) reversed the basic sequence in the following manner. Whole blood was diluted with saline and centrifuged through Ficoll-Hypaque to collect lymphocytes. The remaining cell button, composed of erythrocytes and granulocytes, was resuspended in autologous, single-strength plasma or serum. Granulocytes were then harvested from the resulting cell suspension by dextran sedimentation. The overall cell yields in this system were comparable to those reported by Boyum. Thorsby's protocol offers some practical advantages in that prompt separation of lymphocytes from whole blood minimizes lymphocyte-erythrocyte clumping and potential lymphocyte-granulocyte interactions.

A disadvantage inherent in Thorsby's method is the requirement for fresh autologous plasma during dextran sedimentation. This reduces the potential cell yield available from

an initial volume of whole blood. To maximize cell recovery from the small blood volumes which were used in the present study, a modification of Thorsby's method was devised. In place of fresh autologous plasma, the diluted autologous plasma remaining as a supernatant after lymphocyte flotation was salvaged and used for dextran sedimentation. With appropriate adjustments in the volume and concentration of the sedimentation solutions, the final dextran concentration used by Thorsby (1%) was achieved. The final plasma concentration in the modified system was within the range of 12 to 17%. This was comparable to the plasma concentration (18%) present in a dextran sedimentation system optimized for leukocyte yield (Skoog and Beck, 1956). A pilot study with the modified technique demonstrated that values for granulocyte yield, purity, and viability were comparable to those originally reported by Thorsby (Table 1). The details of the separation are presented below.

Step I. Separation of lymphocytes by Ficoll-Conray flotation. Stock solutions of 9% Ficoll-400 (Pharmacia, Sweden) and 33.4% Conray-400 (sodium iothalamate; Mallinckrodt, USA) were prepared in distilled water and sterilized by suction through 0.22 μ filter units (Falcon Plastics, USA). The solutions were stored at 4° C. A working solution of specific gravity 1.077 was prepared by mixing 10 parts Ficoll stock with 24 parts Conray stock.

Diluted blood in aliquots of 10 ml was carefully layered over 3 ml Ficoll-Conray working solution in 15 ml conical cen-

Mean granulocyte harvest/ml whole blood (10 ⁶ cells)	Cell yield (%)	Purity of granulocyte preparation (%)	RBC:WBC ratio	Viability (%)	
Modified technique	2.04 ± 0.39	49.49 ± 9.89	96.84 ± 1.44	1.91 ± 0.67	98.35 ± 1.91
Thorsby technique	--	50	97.99	1.0 - 5.0	98 - 100

Table 1. Comparison of modified technique with original Thorsby technique for isolation of granulocytes.

In the modified technique, salvaged autologous plasma was used during dextran sedimentation in place of fresh autologous plasma or serum. Solution volumes and concentrations were adjusted to optimize the final dextran and plasma concentrations. Granulocyte counts were performed in an Improved Neubauer Counting chamber (25 trials). Cell yield was calculated from independently obtained white cell counts and differentials (13 trials). Purity of the granulocyte preparations was assessed by examination of May-Giemsa stained smears (25 trials). RBC contamination was determined concomitantly with granulocyte counts (25 trials). Viability was determined by trypan blue dye exclusion (10 trials). Values obtained for the Thorsby technique are reproduced from Vox Sanguinis 13: 194-206, 1967.

trifuge tubes. The tubes were spun at 400 g for 20 minutes at 4° C. After centrifugation, diluted plasma was present as a supernatant at the top of each tube; erythrocytes and granulocytes were found as a cell pellet within the Ficoll-Conray layer at the bottom. Lymphocytes were suspended in a turbid ring at the interface between the plasma and the Ficoll-Conray layers.

With a sterile Pasteur pipette, approximately 5 ml of plasma supernatant was aspirated and transferred to a sterile glass tube. The lymphocytes were similarly collected and used in other studies. The remaining plasma and Ficoll-Conray solutions were withdrawn and discarded. Special care was taken to avoid aspiration of the granulocyte-rich cell layer present at the upper edge of the cell pellet.

Step II. Isolation of granulocytes by dextran sedimentation. Dextran T-500 (MW 500,000; Pharmacia, Sweden) was dissolved in HBSS, pH 7.2, to give a 3% w/v solution. To adsorb granulocyte toxins which may be present in some dextran lots (Martin and Green, 1958), the solution was passed through 5 mm of compacted activated charcoal. Gross impurities were cleared by filtration through a #47 Whatman filter and the solution was then sterilized by suction through a 0.22 μ filter unit. The sterilized solution was stored at 4° C.

The RBC-granulocyte pellet remaining at the bottom of each centrifuge tube was resuspended with autologous salvaged plasma and 3% dextran solution to give a 1:1:1 ratio of pellet, salvaged plasma, and dextran solution. With a sterile wide-tipped Pasteur pipette, the contents of each tube were thoroughly mixed.

Special attention was given to the removal of bubbles and foam that appeared during the mixing process, as persistence of these markedly increased erythrocyte contamination of the granulocyte-rich supernatant. The tubes were placed on a 60° incline and transferred to a 4° C. refrigerator for 30 minutes of sedimentation. In a series of preliminary trials, it was found that shorter sedimentation periods gave higher granulocyte yields, but also resulted in unacceptable levels of erythrocyte contamination (RBC:WBC ratio greater than 10:1). Longer periods of sedimentation reduced erythrocyte contamination as well as granulocyte yield. Similar observations have been reported by Skoog and Beck (1956) and by Klein and coworkers (1958). In the present study, a 30 minute sedimentation period provided an acceptable compromise between erythrocyte contamination and leukocyte loss. This choice was particularly convenient in that a sharp supernatant-erythrocyte interface formed after approximately 25 minutes, and thus served as a good visual reminder of near completion of the sedimentation process.

The granulocyte-rich supernatant was collected with a sterile Pasteur pipette. The cells were washed twice by centrifugation through 10 ml HBSS at 250 g for 10 minutes at room temperature. No more than two washes were performed at this step, as granulocytes subjected to repeated centrifugation often showed microscopic clumping of cells. Once present, cell clumping could not be reversed by vigorous agitation, and preparations containing clumped cells displayed markedly decreased migration on agarose plates. Granulocyte preparations

exhibiting cell clumping were not considered suitable for use.

Absolute and differential cell counts

Autologous washed cells were pooled and resuspended to a total volume of 2 ml in HBSS. Two drops of each cell suspension were individually counted in the central square of an Improved Neubauer Counting Chamber and the average cell harvest (granulocytes recovered/ml whole blood) was calculated. RBC contamination was determined concomitantly by duplicate erythrocyte counts.

To assess mononuclear cell contamination, one drop of granulocyte suspension was mixed with one drop of heat-inactivated fetal calf serum (GIBCO, USA) and a drop of the resulting solution was placed on a clean slide and gently spread over a 1 to 2 square cm surface area with the tip of a pipette. Great care was taken in spreading, as the washed cells seemed to have fragile membranes which ruptured easily when spread by conventional techniques. The slide preparations were air-dried at room temperature and then fixed by 30-second immersion in ice-cold 1:1 acetone and distilled water. The fixed preparations were air-dried and stained for 30 seconds in full-strength May-Grünwalds-Lösung (Merck), 1 minute in half-strength May-Grünwalds-Lösung, and 5 minutes in Giemsa (Merck). Adequate staining of nuclei and cytoplasm was achieved with this method, but cytoplasmic granules did not stain well in all preparations and the sub-populations of basophils and eosinophils were not always distinguishable from each other or from PMN leukocytes. Prior exposure of the cells to heparin may have been responsible

for this difficulty. A differential count of granulocytes and mononuclear cells was made by examination of 200 stained cells under oil immersion.

For calculation of cell yield, routine WBC counts and differentials were performed on whole blood specimens by the Hematology Clinic. The theoretical granulocyte yield was calculated from Clinic data as the product of the total white cell count and the percent PMN leukocytes. Percent cell yield was taken from the ratio of actual granulocyte harvest (corrected for mononuclear cell contamination) to theoretical granulocyte yield. Overestimation of cell yield by eosinophils and basophils which may have been indistinguishable from PMN leukocytes because of sub-optimal staining of cytoplasmic granules was estimated as 3%.

Viability testing

Trypan blue (Chroma-Gesellschaft, Germany) was diluted in HBSS to make a 0.5% w/v solution and filtered through #47 Whatman paper to remove gross impurities. One drop cell suspension was mixed with one drop trypan blue, and one drop of the resulting solution was placed in an Improved Neubauer Counting Chamber. After 5 minutes, 200 cells were inspected. Cells which excluded dye were counted as viable; those showing diffuse cytoplasmic staining or dark nuclear staining were counted as non-viable.

Agarose plate preparation and use

Medium constituents. Nutrient agarose medium containing

1% agarose (tissue culture-1 grade; Nakarai Chemicals, Japan), single-strength Tissue Culture Medium 199 (Nissui Seiyaku, Japan), 20% horse serum (GIBCO, USA), 100 IU penicillin G/ml and 100 µg streptomycin/ml (Meiji Chemicals, Japan) was prepared by the method of Clausen (1971). In addition, colchicine (Lilly, USA) and vincristine sulfate (Oncovin; Lilly, USA) were added to give final drug concentrations of 10^{-9} to 10^{-5} M. Special attention was given to the pH of the medium, which required adjustment with approximately 0.5 mg NaHCO_3 /ml to achieve a final value of 7.2 .

Plate preparation. Molten agarose medium at 45° C. was delivered in 9 ml aliquots into 5.5 cm diameter sterile plastic petri dishes (Falcon Plastics, USA) and allowed to solidify at room temperature. The plates were then hardened for 1 hour at 4° C. A sharp metal template with a 2 mm diameter bore was attached to a low vacuum and used to cut holes in the hardened agarose. The plates were then placed in a humidified 5% CO_2 chamber at 37° C. and allowed to equilibrate for at least 2 hours. Plates containing colchicine were protected from direct light during preparation to minimize the formation of the inactive photoisomer, lumicolchicine (Ertel and Wallace, 1970). Colchicine stock solutions were stored in foil-wrapped glass containers.

To perform time-course studies of PMN leukocyte migration, the advancing edge of cell movement was marked along a specially devised set of lines. After holes were cut in the hardened agarose, the undersurface of each plastic plate was inscribed with a hexaxial reference system, as shown in Figure 1. The tip of a #23 hypodermic needle and a transparent glass slide were

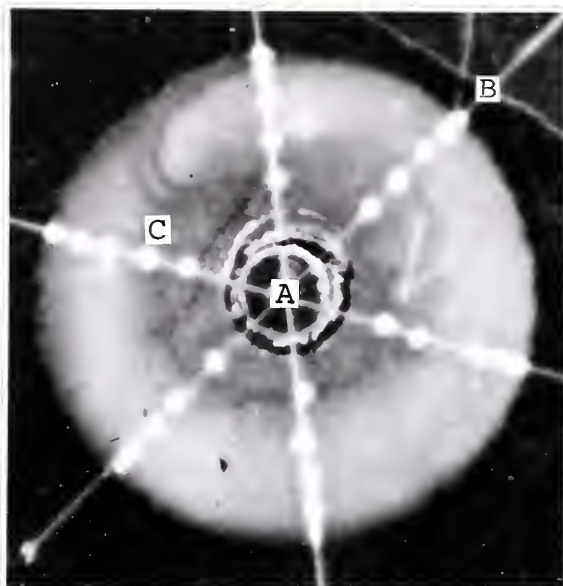


Figure 1. Fixed and stained agarose plate migration pattern of human PMN leukocytes, with hexaxial reference system.

Agarose plates were prepared by the method of Clausen, with the addition of colchicine and vincristine to give drug concentrations of 10^{-9} to 10^{-5} M. Central wells were cut with a 2 mm diameter template and charged with 2×10^6 PMN leukocytes. The plates were incubated for 18 hrs at 37° C in a humidified 5% CO_2 chamber. The central and peripheral migration patterns were studied for evidence of drug effects.

A hexaxial reference system was drawn on the outer surface of the plates for time-course studies of cell migration. At selected intervals, the plates were inspected and the advancing border of cell migration was marked. At the completion of the incubation period, the radii of migration at each time interval were measured and averaged.

A. Central well. B. One component of the hexaxial reference system. C. Mark indicating the position of the advancing cell border at a selected time interval.

Magnification 10X.

used. Plates were carefully checked after inscription of the lines to be certain that spacing was uniform and that the origin of the 6 resultant radii was located at the center of the agarose well. If these conditions were not fulfilled, the reference system was not considered satisfactory for use and a new set of lines was drawn over another agarose well.

Application of cells to plates. Cells which had been previously washed and counted were pelleted by centrifugation at 250 g for 10 minutes at room temperature. Supernatants were inspected for signs of cell clumping (thread-like material, turbid appearance). If these were present, the preparation was discarded; otherwise, the supernatant was removed by suction through a Pasteur pipette and the tubes were briefly inverted to permit evaporation of excess moisture. Agarose-free medium containing single-strength Tissue Culture Medium 199, 20% horse serum, 100 IU penicillin G/ml, and 100 µg streptomycin/ml was used to resuspend the PMN leukocytes to a concentration of 2×10^8 cells/ml. The resuspension medium was delivered through clean BB constriction micropipettes to insure accuracy.

Moisture which had collected in the agarose wells during equilibration was removed by gentle suction through a fine-tipped pipette. Using a clean constriction pipette for each set of cells, aliquots of 2×10^6 PMN leukocytes in a volume of 10 µl were delivered in duplicate to the agarose wells.

Plate incubation and time-course studies. After application of cells, the plates were promptly covered and returned to the 5% CO₂ humidified chamber for 18 hours of incubation at 37° C.

During this period, cells migrated radially in the potential space between the agarose medium and the inner surface of the plates.

For time-course studies of leukocyte migration, plates marked with the hexaxial reference system were briefly withdrawn from the incubator at intervals of 1, 3, 6, 12, and 18 hours. At each time point, the location of the advancing edge was marked along the 6 axes of the reference system. Precision in marking was achieved by the use of an inverted light microscope and a #23 hypodermic needle positioned on a fulcrum. The impression of the needle tip on the undersurface of the plastic petri dish created a permanent record of the progression of PMN leukocyte movement (see Figure 1).

Fixation and staining. After 18 hours, agarose plates were removed from the incubator and covered with 5 ml fixative (3:1 absolute methanol and glacial acetic acid). As the fixative permeated the agarose gel, the phenol red indicator present in the tissue culture medium turned from a pink-orange hue to bright yellow. When the entire thickness of gel was yellow in color (approximately 20 minutes), fixation was considered complete. The agarose medium was detached by freeing the rim of each gel with a thin glass coverslip, and then inverting the plate. The fixed cells remained attached to the plates and were stained with Giemsa for 10 minutes.

Examination of migration patterns. Detailed studies of cell distribution patterns were made under the light microscope. The peripheral edge of cell migration was examined for the

presence of a sharp or irregular border. The distinction between these two types of borders was obvious and did not require formal definition. The central area of cell migration was examined for the presence of a dense or disperse cellular pattern. Dense cellular packing was defined as the presence of a touching or overlapping carpet of cells; disperse cellular packing was defined as the absence of a touching or overlapping carpet. When disperse cellular packing extended outwards for distances greater than 25% of the total migration radius, a pattern of central dispersion was said to exist. When disperse cellular packing was confined to less than 25% of the total migration radius, the pattern was described as one of central density. For graphic displays of cell distribution, stained plates were passed across the 2 mm aperture of a densitometer (Fujiox model FD-AIV).

Measurement of migration patterns. For time-course studies of cell migration, plates were mounted in a portable 35-mm slide projector and viewed against a white background at 20X magnification (planimetry). Measurements were made to the nearest millimeter. The hexaxial reference system provided a set of 6 radii for each migration pattern. Since cells from each individual were studied in duplicate, the average radius of migration for a given time point and drug concentration was taken as the arithmetic mean of 12 migration radii. To facilitate graphical representation, drug effects were expressed in terms of percent migration inhibition (%MI). For a given individual i , percent migration inhibition was defined as

$$\%MI = 100 \cdot \left(1 - \frac{d^{R_{c,t}}}{oR_t}\right)$$

where $d^{R_{c,t}}$ was the average radius of migration in drug-containing medium at a given concentration c and time t , and oR_t was the average radius of migration in parallel drug-free medium at time t . For a given drug and set of individuals studied, a group average, $\%MI_{c,t}$, was defined as

$$\%MI_{c,t} = \frac{\sum (\%MI_i)_{c,t}}{N}$$

where c and t identify the specified drug concentration and time interval, and N represents the number of individuals studied. These average values of percent migration inhibition were plotted as a family of concentration curves, with $\%MI$ as ordinate and time as abscissa.

Statistical analysis

Pattern studies. In studies of central and peripheral migration patterns, the outcomes could be dichotomized in the following manner:

Peripheral edge pattern	Central cell pattern
sharp	dense
not-sharp (irregular)	not-dense (disperse)

The central and peripheral patterns were thus individually suited to two-way independence testing. Since the trial conditions and total number of outcomes were both fixed by experimental design, Fisher's exact test was chosen as the appropriate method of analysis. Central and peripheral pattern frequencies at each drug concentration were compared with the pattern frequencies

observed in drug-free medium. P-values were read directly from a table of the hypergeometrical distribution, prepared for independence testing (Diem and Lentner, 1970). Double-tailed p-values less than 0.05 were considered significant.

Time-course studies. Average radii of migration were calculated as described above. For each time interval and drug concentration, the average radius of migration in drug-free medium was paired by individual with the average radius of migration in drug-containing medium. The paired t-test was then applied. Values of t were computed on a pre-programmed Olivetti calculator, using the mean of sample differences. One less the number of pairs was chosen as df. Single-tailed p-values less than 0.05 were considered significant.

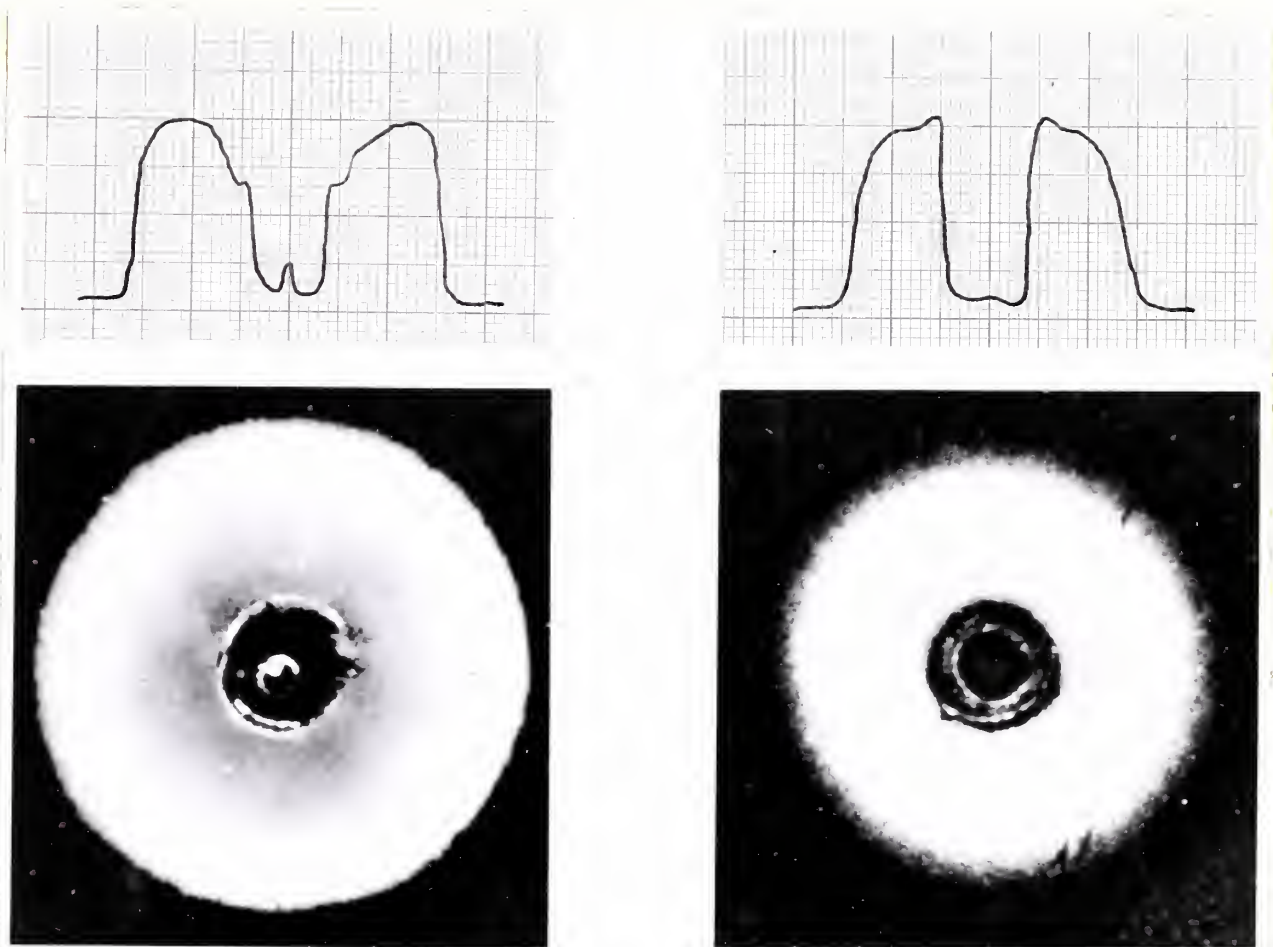
RESULTS

I. The gross migration pattern of human PMN leukocytes is altered at low concentrations of colchicine and vincristine.

Examination of agarose plates with the unaided eye and at low-power magnification revealed significant drug-related changes in migration pattern. On drug-free plates, the peripheral margin of cell migration appeared sharp and well-demarcated, while the coating of cells in the central area of these plates usually appeared thin and uneven. The converse was observed for agarose plates containing colchicine and vincristine at concentrations of 10^{-8} to 10^{-7} M and greater. On these drug-containing plates, the peripheral edge of cell migration was irregular and poorly-defined, while the central area most often appeared to be evenly filled.

Alterations in migration pattern were also evident from densitometer tracings. The sharp peripheral borders seen on drug-free plates appeared as steeply descending lines at the edges of the tracings; the uneven central area of these plates produced gentle tapering of lines in the internal core. In the presence of colchicine and vincristine, these slope configurations were reversed. A gently tapered line was seen at the outer edges of the tracing, while a sharp internal core was present. The densitometer changes thus paralleled the central and peripheral visual findings.

The characteristic patterns of PMN leukocyte migration on



A. Drug-free medium

B. 10^{-8} to 10^{-7} M drug

Figure 2. Drug-induced changes in the agarose plate migration pattern and densitometer profile of PMN leukocytes.

Human PMN leukocytes were applied to the central wells of drug-free and drug-containing agarose plates and incubated for 18 hrs. Fixed and stained plates were photographed under dark-field illumination and scanned with a densitometer.

A. In drug-free medium, migrating cells formed a sharp peripheral margin and a thinly coated central area. The densitometer scan showed abruptly descending lines at the peripheral margins and gradually tapered lines in the central areas of the plate.

B. In medium containing colchicine or vincristine at 10^{-8} to 10^{-7} M, migrating cells formed an irregular peripheral margin and a thickly coated central area. The densitometer tracing showed gradually tapered lines at the peripheral margins and abruptly descending lines in the central areas.

Magnification 10X.

drug-free and drug-containing plates are shown in Figure 2.

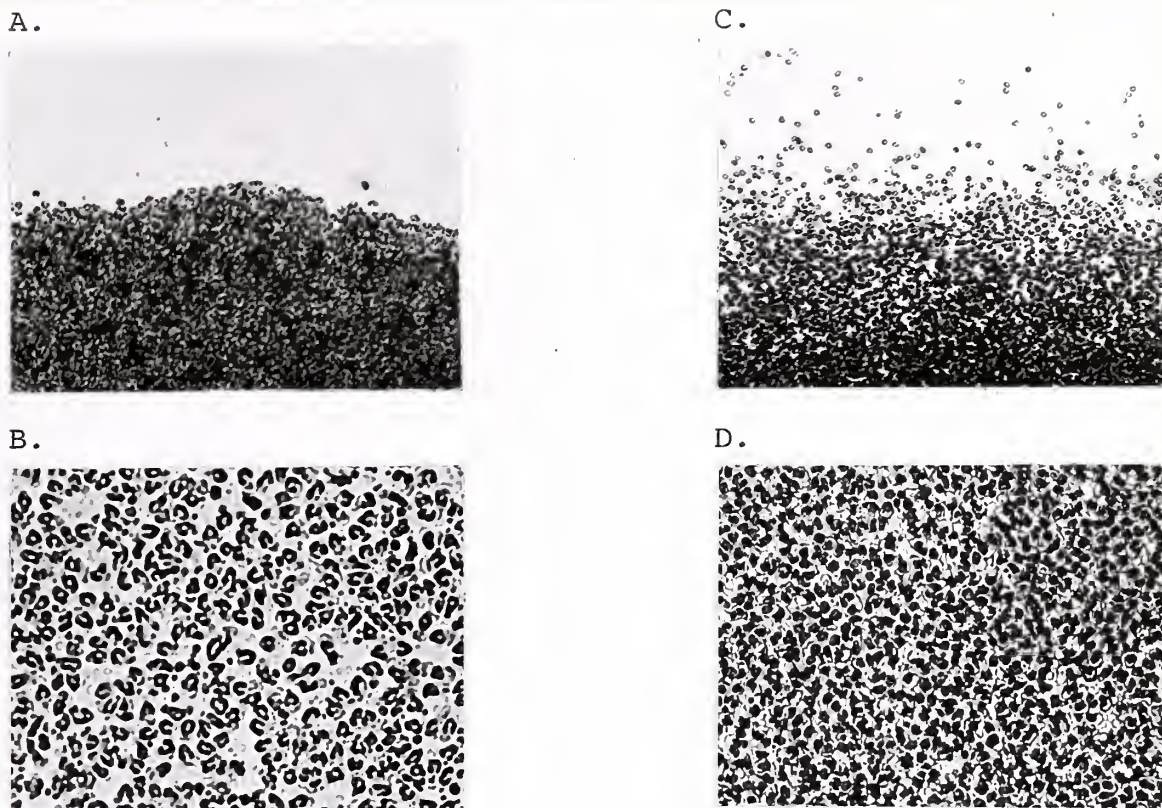
II. Changes in central and peripheral migration patterns are related to changes in the density of cell aggregation.

At high-power magnification, the individual cells present at the center and periphery of the migration plates were visualized. On drug-free plates, the cells at the peripheral margins were closely packed, whereas cells in the central area were loosely aggregated. Converse findings were observed on plates containing colchicine and vincristine at concentrations of 10^{-8} to 10^{-7} M and greater. On these drug-containing plates, cells at the peripheral edge of migration were loosely scattered, while those present in the central area were tightly packed. Photomicrographs showing these differences in cell aggregation are presented in Figure 3.

To ascertain that the observed differences in cell aggregation were not artifacts of fixation and staining, agarose plates were examined under an inverted light microscope immediately after completion of the 18-hour incubation period. The same patterns of peripheral and central cell aggregation were observed in these untreated preparations as in the plates described here.

III. Drug-related changes in leukocyte migration patterns are reproducible, and first appear at concentrations of 10^{-8} to 10^{-7} M.

The reproducibility of central and peripheral changes in migration pattern was investigated at colchicine and vincristine



Drug-free medium

- A. Peripheral edge
- B. Central edge

10^{-8} to 10^{-7} M drug

- C. Peripheral edge
- D. Central edge

Figure 3. Cell aggregation in central and peripheral areas of agarose migration plates.

PMN leukocytes were fixed and stained after 18 hrs migration in drug-free and drug-containing medium. The central and peripheral areas of migration were examined under sufficient magnification to permit resolution of individual cells.

In drug-free medium, the cells forming the peripheral edge were densely packed (A), whereas those present in the central region were loosely aggregated (B). This pattern was reversed in 10^{-8} to 10^{-7} M drug-containing medium, in which the peripheral edge (C) showed loose aggregation of cells and the central region displayed dense cellular packing (D).

Peripheral edge magnification, 160X. Central area magnification, 400X.

concentrations of 10^{-9} to 10^{-5} M. PMN leukocytes from 7 individuals were tested in duplicate on a full series of colchicine-containing plates; leukocytes from 5 individuals were similarly tested with vincristine. Parallel trials in drug-free medium were also performed. The frequency of pattern change at each drug concentration was determined and compared with the results of the drug-free trials. Significance was assessed by applying Fisher's exact test.

In colchicine-containing medium, irregularity of the peripheral margin first appeared at a concentration of 10^{-8} M; in vincristine-containing medium, irregularity first appeared at 10^{-7} M. At the concentrations studied here, change in the peripheral migration pattern occurred as an "all or none" phenomenon. The frequency of peripheral pattern change was statistically significant at colchicine concentrations of 10^{-8} M and greater, and at vincristine concentrations of 10^{-7} M and greater.

In contrast to the abrupt changes seen in peripheral migration pattern, the changes observed in central migration pattern were graduated. In drug-free medium, approximately 30% of all migration patterns demonstrated central density. With rising drug concentration, this percentage also increased, reaching 100% at a colchicine concentration of 10^{-7} M and a vincristine concentration of 10^{-6} M. The frequency of central density was significantly increased at colchicine and vincristine concentrations of 10^{-7} M and greater.

Figure 4 summarizes the observed changes in PMN leukocyte mi-

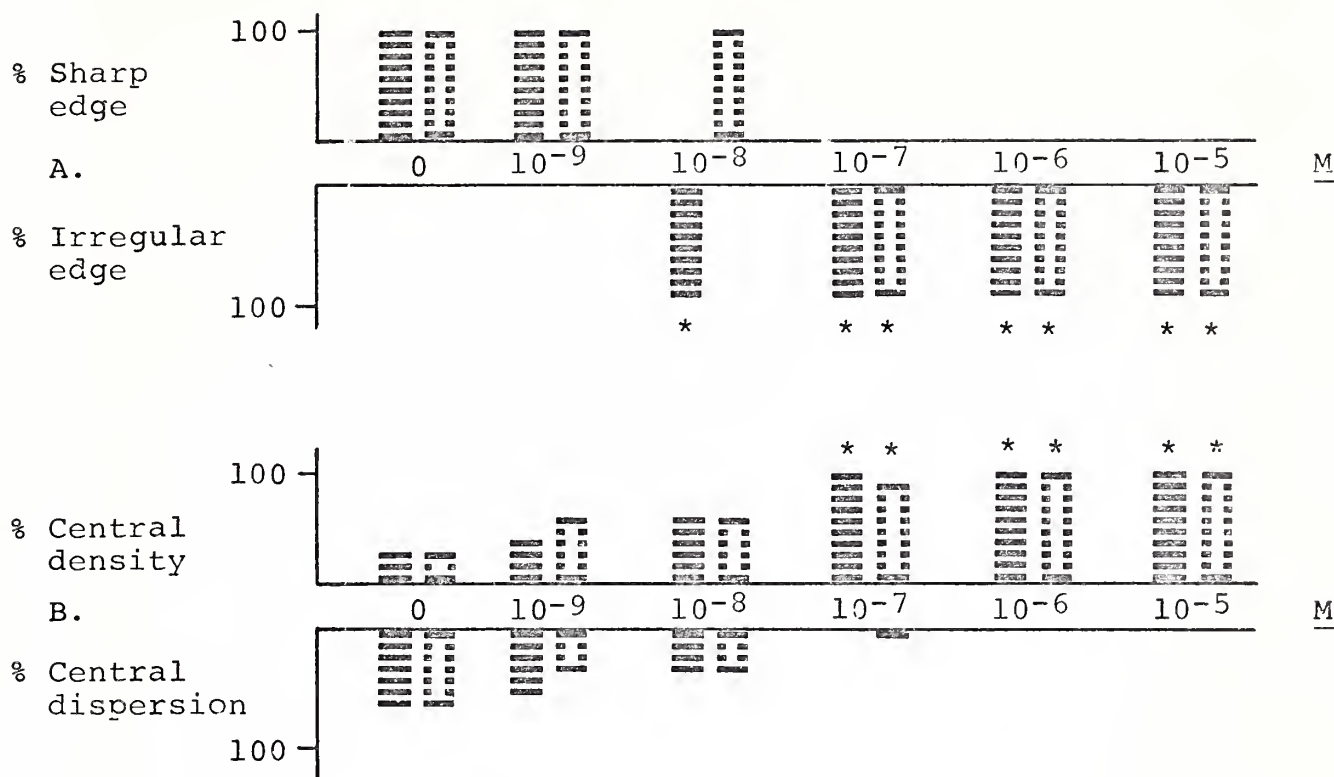


Figure 4. PMN leukocyte migration patterns at varying concentrations of colchicine and vincristine.

The migration patterns of PMN leukocytes were studied at colchicine and vincristine concentrations of 10^{-9} to 10^{-5} M. Cells from 7 individuals were tested in duplicate in colchicine-containing media; cells from 5 individuals were similarly tested with vincristine. A tabulation was made of the central and peripheral distribution of cells at each drug concentration. Findings were expressed as percent of trials showing a specified cell distribution pattern. Significance was assessed by applying Fisher's exact test.

A. Peripheral edge findings. B. Central area findings.

Solid-striped bars represent colchicine trials; open-striped bars represent vincristine trials. An asterisk (*) is used to note drug concentrations at which the observed migration pattern differed significantly ($2p < 0.05$) from that of the drug-free trials.

gration patterns in the presence of colchicine and vincristine at concentrations of 10^{-9} to 10^{-5} M.

IV. Colchicine and vincristine inhibit leukocyte radial migration at drug concentrations of 10^{-8} to 10^{-7} M.

Time-course studies of PMN leukocyte migration were conducted at colchicine and vincristine concentrations of 10^{-9} to 10^{-5} M. Eight individuals were studied in duplicate on a full series of colchicine-containing plates; five individuals were similarly studied with vincristine. Parallel time-course studies in drug-free medium were also performed. For each drug and drug concentration, the average radius of migration was paired by time interval with values from the drug-free trials. Statistical significance was assessed by applying the paired t-test. Drug effects were graphically displayed in terms of percent migration inhibition.

The effect of colchicine on radial migration of PMN leukocytes is shown in Figure 5. The predominant action of the drug was inhibitory, with significant migration inhibition first appearing at 6 hours in 10^{-7} M medium. An interesting feature of the colchicine studies was the variation in drug effect with time. At concentrations of 10^{-9} and 10^{-8} M, a small but significant stimulating effect was found in the first hour; thereafter, no significant stimulation was observed. Additionally, at drug concentrations of 10^{-7} M and greater, migration inhibition showed a tendency to peak at 6 hours. At the 3 and 12 hour intervals, only 4 migration patterns were available for

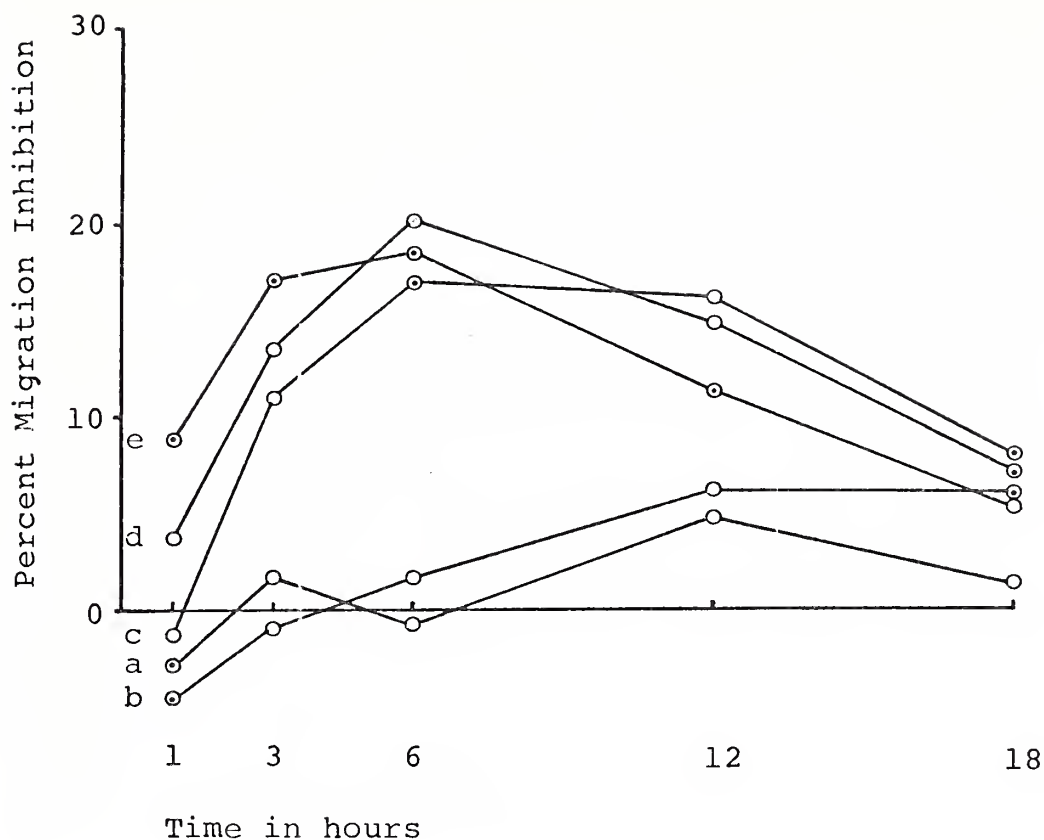


Figure 5. Time-dependent effect of colchicine on PMN leukocyte migration.

At intervals of 1, 3, 6, 12, and 18 hrs, the average radius of cell migration was measured in media containing colchicine at concentrations of (a) 10^{-9} , (b) 10^{-8} , (c) 10^{-7} , (d) 10^{-6} , and (e) 10^{-5} M. For each of the eight individuals studied, parallel measurements in drug-free media were also obtained. Differences between the average radius of migration in drug-containing and drug-free media were expressed in terms of average percent migration inhibition (see text).

To assess the significance of drug-related changes in migration radii, the average radius of migration in drug-free media was matched by individual to the average radius of migration in drug-containing media at each time interval and concentration. The paired t-test was then applied. Significant outcomes ($p < 0.05$) are indicated in the figure by use of a targeted dot (o).

measurement at drug concentrations of 10^{-9} to 10^{-6} M. The sample size was thus too small at these points for accurate t-testing, but the values for migration inhibition were included on the graphs as they were consistent with the observed trends.

Migration inhibition in the presence of vincristine is shown in Figure 6. As in the colchicine trials, the predominant drug effect was inhibitory. A tendency toward peak migration inhibition at 6 hours was also seen. Unlike the colchicine trials, however, there was no significant stimulation of leukocyte migration at low doses. Migration inhibition at vincristine concentrations of 10^{-6} M and greater was far more pronounced than at equimolar concentrations of colchicine.

To explore the possibility of physiochemical deterioration of colchicine and vincristine as a factor in the apparent reversibility of migration inhibition at late time intervals, agarose plates containing 10^{-8} M colchicine and 10^{-7} M vincristine were prepared in the usual manner and stored for 18 hours at 37° C. in a humidified 5% CO_2 chamber. The plates were then charged with leukocytes and returned to the CO_2 chamber for an additional 18 hours of incubation. At the end of this period, the migration patterns were examined and typical irregularities in the peripheral cell edge were found. Since the threshold effect of these drugs was preserved over time, it seemed reasonable to exclude physiochemical decay as a major factor in the decline of migration inhibition beyond the 6 hour interval. It was not possible to make direct comparisons of migration inhibition in fresh vs. aged

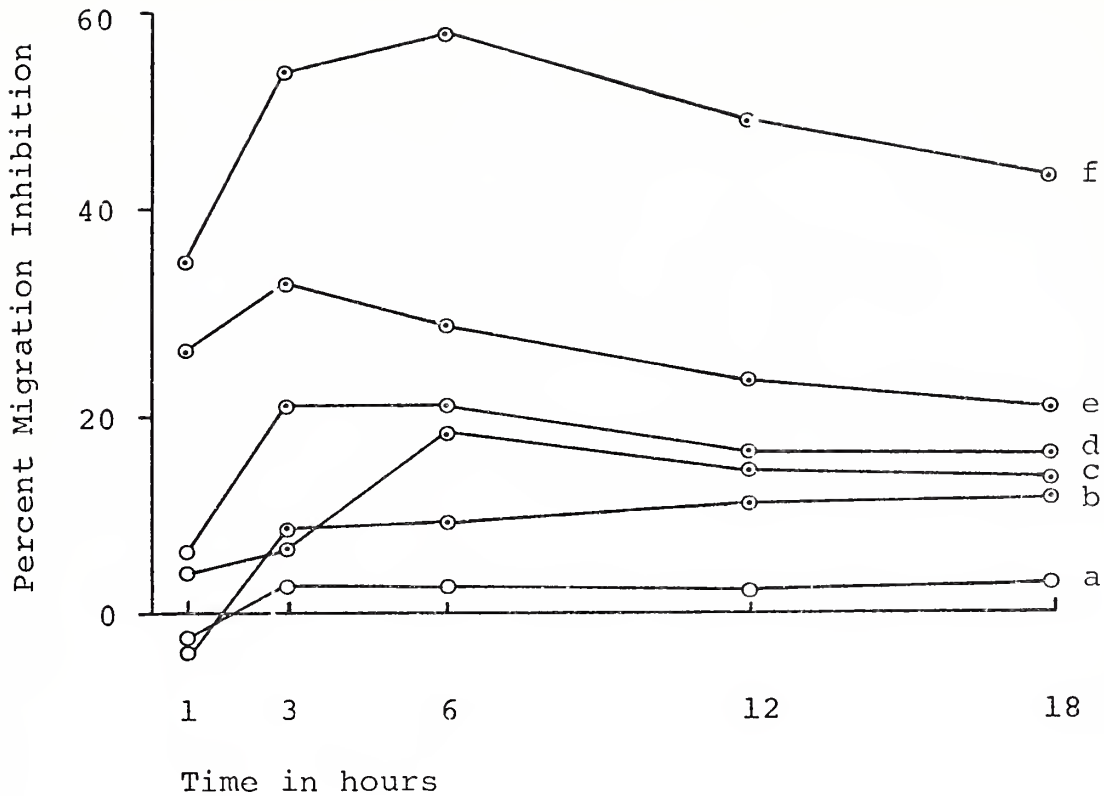


Figure 6. Time-dependent effect of vincristine on PMN leukocyte migration.

At intervals of 1, 3, 6, 12, and 18 hrs, the average radius of cell migration was measured in media containing vincristine at concentrations of (a) 10^{-9} , (b) 10^{-8} , (c) 10^{-7} , (d) 10^{-6} , (e) 5×10^{-6} , and (f) 10^{-5} M. For each of the five individuals studied, parallel measurements in drug-free media were also obtained. Differences between the average radius of migration in drug-containing and drug-free media were expressed in terms of average percent migration inhibition (see text).

To assess the significance of drug-related changes in migration radii, the average radius of migration in drug-free media was matched by individual to the average radius of migration in drug-containing media at each time interval and concentration. The paired t-test was then applied. Significant outcomes ($p < 0.05$) are indicated in the figure by use of a targeted dot (◉).

media, as storage for periods longer than 18 hours was frequently associated with mold growth and dessication.

DISCUSSION

Colchicine and vincristine belong to a class of anti-mitotic agents known as the antitubulins (Margulis, 1973). Physiochemical studies (Borisly and Taylor, 1967; Bryan, 1972) have demonstrated that these drugs act in vitro by binding to tubulin, a protein dimer present in microtubules (Adelman et al., 1968). Evidence has accumulated to suggest that the antitubulins also act on microtubules in vivo. Using polarized light microscopy, Inoué (1952) made an early report of the disruptive action of colchicine on the mitotic spindle present in the oocyte of the marine worm, C. pergamentaceus. Malawista and coworkers (1968) extended these studies to demonstrate the disruptive action of other antitubulins (vinblastine, vincristine, colcemid, podophyllotoxin, and griseofulvin) on the mitotic spindle in the oocyte of P. gouldi. Especially compelling findings have come from electron microscope studies. Microtubules are no longer evident in EM sections of human PMN leukocytes which have been pretreated with colchicine at concentrations of 2.5×10^{-5} M and greater (Malawista and Bensch, 1967). In similar EM studies, pretreatment of leukocytes with vincristine and vinblastine at concentrations of 10^{-5} M has been associated with the appearance of unusual crystalline lattices composed of microtubule-like units (Bensch and Malawista, 1968).

The antitubulins have been reported to alter or inhibit cell mobility in several experimental systems. In mouse peritoneal

macrophages, Bhisey and Freed (1971) noted a loss of gliding movements in cells exposed to colchicine at 10^{-5} M and vincristine at 10^{-6} M. Spooner and coworkers (1971) observed abnormal movement of cultured glial cells at a colchicine concentration of 10^{-6} M. In human PMN leukocytes, Ramsey and Harris (1972) found decreases in the rate of individual cell movement at 10^{-3} M colchicine and 10^{-5} M vincristine. Demecolcine, a colchicine derivative, inhibited chemotaxis of human PMN leukocytes at a concentration of 10^{-8} M in a Boyden chamber study (Bandman et al., 1974). A similar study by Caner (1965) demonstrated decreased chemotaxis of human leukocytes in the presence of 10^{-7} M colchicine. Some workers (Spooner et al., 1971; Ramsey and Harris, 1972) have been hesitant to attribute changes in cell mobility directly to antimicrotubular effects, as near normal or normal locomotion was observed at colchicine concentrations sufficient to disrupt microtubules. It is interesting to note, however, that higher concentrations of antitubulins were required to demonstrate inhibitory effects in experimental systems which required examination of the motion of individual cells. In Boyden chamber experiments, in which the behavior of a cell population was measured, lower concentrations of antitubulins were associated with significant inhibition of movement. The differences may thus be partially attributable to sample size and the sensitivity of study techniques. Chemotaxis itself may be more susceptible to antitubulin effects than simple random migration (Bandman et al., 1974).

Caner's study (1965) occupies an important position in the

literature on the antitubulins, as it affords plausible evidence for an anti-inflammatory action of colchicine in gout. At present, the pathogenesis of acute gouty arthritis is understood as a process requiring the presence of monosodium urate crystals in the synovial fluid (McCarty and Hollander, 1961; Faires and McCarty, 1961), phagocytosis of urate crystals by PMN leukocytes (McCarty, 1962), and acceleration of the inflammatory cycle by the metabolic products of the inflammatory reaction (Seegmiller et al., 1962a). Pharmacological studies have shown that a therapeutic intravenous injection of colchicine produces a transient plasma level of approximately 10^{-8} M (Wallace et al., 1970). Additionally, there is some preliminary evidence to suggest that leukocytes sequester colchicine intracellularly at drug levels of approximately 10^{-7} M for at least 24 hours following a therapeutic intravenous dose (Ertel and Wallace, 1974). Colchicine inhibits phagocytosis (Seegmiller et al., 1962b; Malawista and Bodel, 1966), lysosomal release (Rajan, 1966), and cellular respiration (Malawista and Bodel, 1966), but these effects have not been demonstrated at concentrations as low as those detected after a therapeutic dose. Caner's finding of decreased PMN leukocyte chemotaxis at 10^{-7} M thus provides at least one possible mechanism for colchicine action which is consistent with present distribution studies. The numerous sol-to-gel transformations required during cell chemotaxis may render the PMN leukocyte especially susceptible to colchicine effects (Malawista, 1965).

The present study also provides evidence for antitubulin

effects on cell locomotion. In the presence of colchicine and vincristine at concentrations of 10^{-8} to 10^{-7} M, the central and peripheral patterns of cell distribution are altered, and time-course studies of cell migration reveal an inhibitory effect. The finding of migration inhibition at a colchicine concentration of 10^{-7} M is of special interest in that it confirms Caner's observation with an independent technique and provides further evidence for colchicine action at therapeutic levels.

Colchicine and vincristine produced generally similar effects on PMN leukocyte migration in the agarose plate system. A striking difference was noted, however, in the degree of migration inhibition produced at 10^{-5} M. At this concentration, the peak value for migration inhibition in the presence of colchicine was approximately 20%, whereas the peak value with vincristine was nearly 60%. Colchicine binds tubulin in a weak and reversible manner (Bryan, 1972; Taylor, 1965), while the binding of vincristine to tubulin is comparatively strong (Wilson, 1970). The two antitubulins also occupy different binding sites on the tubulin dimer (Bryan, 1972). The differences in migration inhibition may thus be related to physiochemical dissimilarities in the interaction of these compounds with microtubular substructures.

A noteworthy aspect of the time-course studies was the tendency for migration inhibition to peak after 3 to 6 hours of incubation. Creasy and Chou (1968) reported that colchicine enters sarcoma 180 cells very rapidly, reaching a maximum intracellular concentration at 20 minutes. In human leukocytes, Ertel

and Wallace (1971) found peak drug levels 10 minutes after administration of an intravenous dose. Intracellular binding to microtubular protein, however, apparently proceeds at a slower rate, requiring 4 to 5 hours to reach a maximum in human KB cells incubated in the presence of 10^{-5} M colchicine (Taylor, 1965). A reasonable synthesis of these observations is that colchicine distribution occurs first as a rapid phase of cellular uptake and second as a slower phase of intracellular equilibration with microtubular binding sites. The time-course observations presented here suggest that colchicine effects may proceed in parallel with the events of the second distribution phase.

In studies of the frog melanocyte, Malawista (1965) demonstrated that colchicine effects were a function of drug concentration and length of exposure. It was thus initially anticipated that time-course studies of colchicine and vincristine would show most pronounced migration inhibition at the more distant time intervals. This, however, was not observed. A possible explanation may be exhaustion of drug stores in the central region of the agarose medium, caused by repeated passage of cells through this area. Cells which leave the central well at an early time pass through the central region when its drug content is highest and thus receive the greatest exposure. These cells probably experience the greatest degree of migration inhibition and may well be responsible for the dense cellular carpet which appears in the central region of drug-containing plates. Conversely, cells which leave the central well at later times may

experience little drug exposure during the first few millimeters of travel. This population of less affected cells may therefore account for the diminution of migration inhibition which occurs at later time intervals. They may also comprise the population of farthest-moving cells which form the irregular border on drug-containing plates. Other mechanisms which may contribute to the diminution of migration inhibition are reversibility of drug-binding and cell repair mechanisms. The irregular peripheral margins and dense central regions may also represent innately resistant and susceptible sub-populations of PMN leukocytes.

An unexpected finding in the present study was the small but significant stimulation of PMN leukocyte migration which appeared during the first hour of incubation at colchicine concentrations of 10^{-9} and 10^{-8} M. Significant stimulation did not occur at any other time interval or concentration of colchicine, or in any trial with vincristine. Contamination seems unlikely as an explanation, as several fresh preparations of colchicine were used during the study. The purity of the pharmaceutical preparation itself has been previously established (Wallace et al., 1970). No similar reports have been encountered in the literature, and the observation presently remains as an unexplained curiosity.

Leukocyte locomotion in most experimental systems is described as either chemotactic or random. The Boyden chamber technique is generally regarded as the standard method for studying chemotaxis (Keller et al., 1975). Commonly accepted methods for assessment of random migration include the capillary tube tech-

nique of Ketchel and Favour (1955) and leukocyte penetration of a Millipore filter in the absence of a chemotactic stimulus in the lower compartment of a Boyden chamber. Recently, random walk models for independent cell movement have been described and applied to experimental systems. Gail and Boone (1970) reported that the motion of individual mouse fibroblasts in tissue culture conformed with statistical expectations for random particle motion on a plane; similar results were obtained by Peterson and Noble (1972) in a study of PMN leukocyte movement using a modified slide and coverslip chamber. The latter investigators suggested that it may be inaccurate to refer to methods such as the capillary tube technique and Millipore filter penetration as true measures of random cell movement unless appropriate statistical tests can be applied.

An interesting consideration raised by the present study is whether the agarose plate technique involves PMN leukocyte chemotaxis or random migration. An element of outward chemotaxis must exist by virtue of design, as the presence of a central well creates a local deficit of nutrient medium. Exhaustion of medium by repeated passage of cells through the central area may also contribute to an outward chemotactic gradient. These two factors probably account for the dispersion of cells observed in the central area of drug-free plates. Factors which would potentially favor random migration include suspension of cells in nutrient medium of the same composition as that of the gel, and the homogeneity of the medium present in the plates. Strict statistical considerations, however, eliminate random migration as

a real consideration. If migration were truly random, the density of cell distribution would be expected to decay from the center to the periphery. Simple inspection of drug-free plates shows that this condition does not obtain.

In sum, PMN leukocyte migration in the agarose plate technique probably involves some degree of outward chemotaxis. This chemotactic effect is inherent in the design of the technique and is difficult to quantitate. At present, an adequate term for leukocyte migration in the agarose plate system might be a purely descriptive one. "Radial migration" seems satisfactory for this purpose.

The technique described here might be well suited for additional studies. A logical extension of the present work would be an evaluation of agarose plate migration characteristics of PMN leukocytes from individuals receiving therapeutic doses of colchicine and vincristine. Concomitant determination of the intracellular concentration of colchicine in the PMN leukocytes of these individuals would represent an important adjunct study, as the study performed by Ertel and Wallace (1974) only measured colchicine concentration in the white blood cell population as a whole and did not include any functional correlates. Investigation of other well-known antitubulins would also be of interest. Mobilization of the marginal pool of leukocytes with epinephrine (Samuels, 1951) or the marrow reserves with corticosteroids (Bishop et al., 1968) would provide important cell populations for comparative study.

An interesting attribute of the agarose plate technique is

that the migration pattern is spread two-dimensionally along a gradient potentially composed of cells with the most active migration at the periphery and least active migration in the central area. Differential staining techniques may reveal some histochemical distinctions in the populations spread along this gradient. The effect of phagocytosis upon cell migration might be assessed by mixing cells which have ingested a readily identifiable material with an autologous set of control cells, and then mapping the distribution of each group on the resulting migration pattern.

SUMMARY

The agarose plate technique of Clausen was modified by the addition of colchicine and vincristine to the nutrient agarose gel, and by the use of a hexaxial reference system. Studies of human PMN leukocyte migration with this system revealed three drug-related changes:

1. Loss of sharpness at the peripheral margin of cell migration, occurring at a colchicine concentration of 10^{-8} M and a vincristine concentration of 10^{-7} M.
2. Change in central cell aggregation from a disperse to a dense pattern, occurring with a significantly increased frequency at colchicine and vincristine concentrations of 10^{-7} M.
3. Inhibition of radial cell migration, most pronounced at 3 to 6 hours, occurring at a colchicine concentration of 10^{-7} M and a vincristine concentration of 10^{-8} M.

The findings of an altered agarose plate migration pattern and PMN leukocyte migration inhibition at 10^{-8} to 10^{-7} M are of special interest in that a therapeutic intravenous dose of colchicine produces a transient plasma level, and possibly a more prolonged leukocyte level, within the same concentration range. These results may thus provide some support for the hypothesis that the anti-inflammatory action of colchicine in acute gouty arthritis is attributable in part to altered PMN leukocyte mobility.

The agarose plate technique may provide a useful tool for the study of PMN leukocyte migration in that it potentially distributes cells along a gradient of migratory vigor. The factors influencing cell migration in this system are discussed and possible applications are considered.

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