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THE EFFECT OF ANTIMICROBIAL AGENTS ON LEUKOCYTE CHEMOTAXIS

NANCY B. ESTERLY, M.D., NANCY L. FUREY, M.D., AND LILLIAN E. FLANAGAN, B.S.

Division of Dermatology, Department of Pediatrics, Michael Reese Hospital and Medical Center, Chicago, Illinois, and
Department of Dermatology, Northwestern University School of Medicine,
Chicago, Illinois, U.S.A.

The effects of several chemotherapeutic agents on the chemotaxis of human leukocytes were studied in an *in vitro* system using a Sykes-Moore chamber and a double-filter technique. Chemotactic factor was generated by the interaction of normal human serum and zymosan. At concentrations comparable to and below therapeutic blood levels, tetracycline HCl, erythromycin base and clindamycin HCl were all inhibitory, causing marked suppression of leukocyte chemotaxis and slight reduction of random migration. Penicillin G-Na, dapsone, and sulfapyridine did not alter white cell motility at the concentrations of drug tested. It is postulated that the capacity of some of these agents to inhibit leukocyte chemotaxis may account, in part, for their efficacy in inflammatory skin diseases such as acne vulgaris.

Oral administration of certain antibiotics and, more recently, topical application of these antibiotics have been considered effective therapeutic measures for patients with acne vulgaris. Success with these agents has been attributed, in part, to the reduction in numbers of normal cutaneous flora, particularly *Corynebacterium acnes*, and to interference with the action of bacterial lipases on sebum triglycerides [1-3]. Thus, by reducing the populations of specific organisms and by partially suppressing the formation of free fatty acids, the natural progression of acne lesions from comedones and papules to pustules and cysts theoretically might be interrupted. Although numerous investigators have documented the effects of antibiotics on cutaneous flora and on quantitative changes in surface lipids, not all of these studies have demonstrated a direct relationship between administration of drug and reduction in bacterial counts, alteration in composition of surface lipids, and clinical improvement of skin lesions [4-13]. Some of the discrepancies may be explained by differences in methodology; however, the participation of additional undescribed factors may account for the therapeutic effect.

There is evidence in the literature to suggest that certain antimicrobial agents may suppress leukocyte chemotaxis [14,15]. Since inflammatory acne lesions, which are most susceptible to antibiotic therapy, are the result of an influx of polymorphonuclear leukocytes [16], it seemed reasonable to consider the hypothesis that suppression of leukocyte chemotaxis might account, in part, for the efficacy of these agents. The present study represents an attempt to measure the effects of several of these agents on leukocyte chemotaxis in

an *in vitro* system, using concentrations of antibiotics below, within, and above the range of therapeutic serum levels. Also included in these assays are two chemotherapeutic agents (dapsone and sulfapyridine) that are prescribed frequently for a variety of inflammatory skin diseases.

METHODS

Cell Preparations

On the day of assay, venous blood was collected from healthy human volunteers in a heparinized syringe. One ml of Plasmagel (HTI Corporation, Buffalo, N.Y.) was added for each 10 ml of blood collected, and the syringe was incubated at 37°C in an inverted position for 2 hr to permit sedimentation of cells. The leukocyte-rich plasma was separated from the erythrocytes and centrifuged at 900 rpm for 10 min. The cell pellet was suspended in minimal essential media (MEM) (Grand Island Biological Company, Grand Island, N.Y.) with 10% fetal calf serum (FCS) (Reheis Chemical Corporation, Phoenix, Ariz.), washed twice with MEM and resuspended in 5 ml of MEM-FCS for counting. The final leukocyte concentration was adjusted to 2.5×10^6 /ml by dilution with MEM-FCS, and a cell viability of at least 99% was established, using the trypan blue dye exclusion method.

Chemotactic Factor

Chemotactic factor was generated by incubation of fresh or frozen (-70°C) normal human serum with zymosan (Sigma Chemical Co., St. Louis, Mo.) in a concentration of 2 mg/ml for 1 hr at 37°C. The zymosan was removed by centrifugation, and the serum was heated to 56°C for 30 min to terminate the reaction [17].

Preparation of Antibiotics

The following drugs were tested in chemotaxis assays: erythromycin base (Abbott Laboratories, North Chicago, Ill.), tetracycline HCl (Lederle Laboratories, Pearl River, N.Y.), clindamycin HCl (Upjohn Company, Kalamazoo, Mich.), penicillin G-Na (Upjohn Company, Kalamazoo, Mich.), dapsone (Ayerst Laboratories, New York, N.Y.), and sulfapyridine (Lilly Laboratories, Indianapolis, Ind.). Stock solutions of each antibiotic were prepared in MEM and appropriate amounts added to achieve the desired concentration in the chamber. High concentration stock solutions of sulfapyridine and dapsone were prepared in absolute ethanol and diluted with MEM. Tetracycline HCl and erythromycin base were assayed in concentrations of .01 µg/ml, 1 µg/ml, 10 µg/ml, and 300 µg/ml. Clindamycin HCl was added in concentrations of .2 µg/ml, 2 µg/ml, 20 µg/ml, and 200 µg/ml; penicillin G-Na in concentrations of .01 µg/ml, .1 µg/ml, 10 µg/ml, and 100 µg/ml. Sulfapyridine was tested at 50 µg/ml and 100 µg/ml and dapsone at 25 µg/ml and 50 µg/ml. Antibiotics were added to the lower (attractant) compartment of the chamber in the first set of experiments. In a second set of experiments, the antibiotic was added to the leukocyte suspension in the upper compartment. Chemotactic assays with each concentration of drug were performed in duplicate or triplicate.

Chemotaxis Assays [18]

The chambers used for the assays were of the Sykes-Moore type (Bellco Biological Glassware and Equipment, Vineland, N.J.) [19]. Two Millipore filters (Millipore Corporation, Bedford, Mass.) 25 mm in diameter and 3 µ pore size were inserted between the two compartments and held in place by a rubber gasket [20]. The chamber was sealed at both ends by a 25-mm circular glass cover slip (Rochester Scientific Co., Rochester, N.Y.). Injection of materi-

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Abbreviations:

FCS: fetal calf serum

MEM: minimal essential media

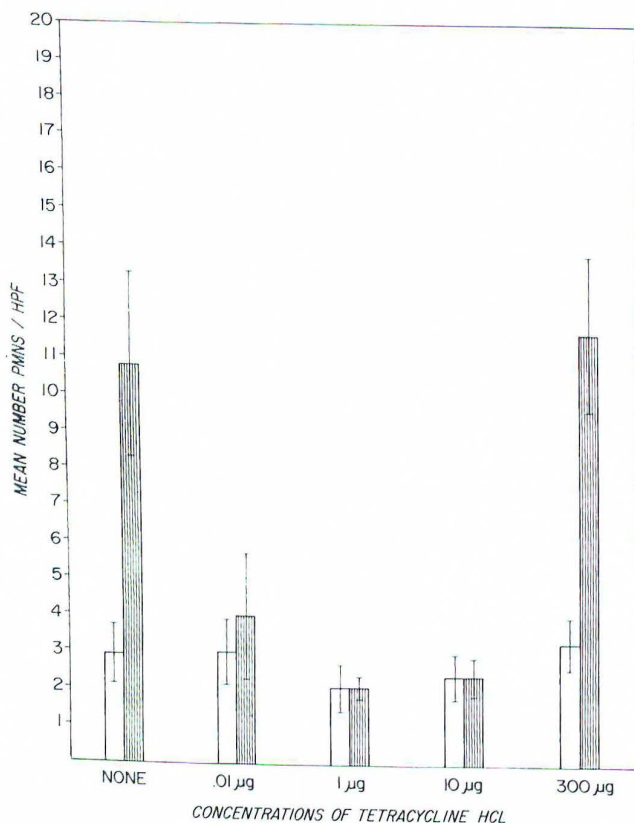


FIG 1. Chemotactic activity of human leukocytes with different concentrations of tetracycline HCl added to the lower compartment of the chamber. Clear bars represent the mean of 8 assays (± 1 SD) for random migration of leukocytes. Dark bars represent mean values (± 1 SD) for neutrophil chemotaxis.

als was made with a 25 gauge needle through the chamber ports. Placement of a second 25 gauge needle in the opposite port allowed air to escape during filling, resulting in a bubble-free chamber. One-half ml of leukocyte suspension (1.25×10^6 cells) was added to the upper compartment, and an 0.8 ml aliquot of fluid (1 part human serum with or without zymosan plus 1 part MEM-FCS with or without drug) was added to the lower chamber. In the second set of experiments, appropriate concentrations of drug in MEM were added to the leukocyte suspension in the upper compartment. The chambers were incubated at 37°C for 3 hr. The filters were removed, fixed and stained in an ethanol, hematoxylin, ethanol, xylene sequence, and mounted with Permount on slides for counting. The cells on each bottom filter were counted in 30 random fields using a 10 \times ocular and 40 \times objective.

All suspensions were prepared in a sterile fashion, and the chambers were autoclaved prior to use. No problems with bacterial growth were encountered. Cell viability was checked again with trypan blue dye at the termination of each experiment and remained greater than 99% in all chambers.

Statistical analysis was performed using Student's *t*-test.

RESULTS

The mean values ± 1 SD for the chemotaxis assays with tetracycline-HCl are depicted in Fig 1. The mean value for random migration of cells from 8 human volunteers (8 separate assays) was 2.9 ± 0.8 cells/hpf, whereas the mean with chemotactic factor was 10.8 ± 2.5 cells/hpf ($p < .0005$). When tetracycline was added in concentrations of .01 µg/ml, 1 µg/ml and 10 µg/ml, there was striking suppression of chemotaxis ($p < .0005$), most marked at concentrations of 1 µg and 10 µg/ml. Minimal inhibition of random migration was observed at the same concentrations ($p < .025$ with 1 µg/ml of drug;

other values not significant). However, at a concentration of 300 µg/ml, there was no effect on chemotaxis (mean of 11.7 ± 2.1 cells/hpf) or random migration (mean of 3.3 ± 0.7 cells/hpf).

The effects of erythromycin base on leukocyte chemotaxis as shown in Fig 2 were similar to those of tetracycline. The mean value for random migration of cells in 8 assays was 3.0 ± 0.4 cells/hpf and that for chemotaxis 14.0 ± 1.0 cells/hpf ($p < .005$). Concentrations of .01 µg/ml, 1 µg/ml, and 10 µg/ml of erythromycin caused significant suppression of chemotaxis ($p < .0005$ for all concentrations) and had a minimal effect on random migration ($p < .01$ for .01 µg/ml; $p < .005$ for 1 µg/ml and 10 µg/ml). Erythromycin at 300 µg/ml failed to inhibit chemotaxis or random migration, and the values obtained with this concentration of drug were not significantly different from the mean control values (3.0 ± 0.4 cells/hpf for random migration and 14.0 ± 1.1 cells/hpf for chemotaxis).

Clindamycin HCl also caused inhibition of chemotaxis at concentrations of .2 µg/ml, 2 µg/ml, 20 µg/ml, and 200 µg/ml as demonstrated in Fig 3. The mean value for random migration was 3.2 ± 0.5 cells/hpf and that for chemotaxis 12.9 ± 1.9 cells/hpf ($p < .0005$). There was significant suppression of chemotaxis at all concentrations ($p < .0005$) and a minimal effect on random migration ($p = .01$ at .2 and 2 µg/ml; $p < .1$ at 20 µg/ml) with the lower concentrations of drug.

In contrast, penicillin G-Na in concentrations of .01 µg/ml, .1 µg/ml, 10 µg/ml, and 100 µg/ml failed to inhibit leukocyte motility (Fig 4). Neither sulfapyridine in concentrations of 50 µg/ml and 100 µg/ml nor dapsone in concentrations of 25 µg/ml and 50 µg/ml had any perceptible effect on leukocyte movement under the conditions of the assay (Fig 5).

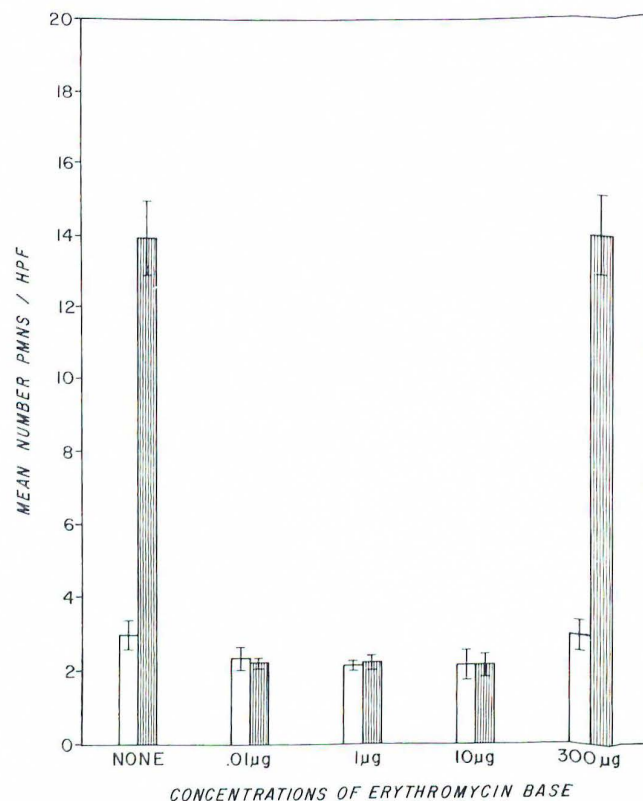


FIG 2. Chemotactic activity of human leukocytes with different concentrations of erythromycin base added to the lower compartment of the chamber. Clear bars represent the mean of 8 assays (± 1 SD) for random migration of leukocytes. Dark bars represent mean values (± 1 SD) for neutrophil chemotaxis.

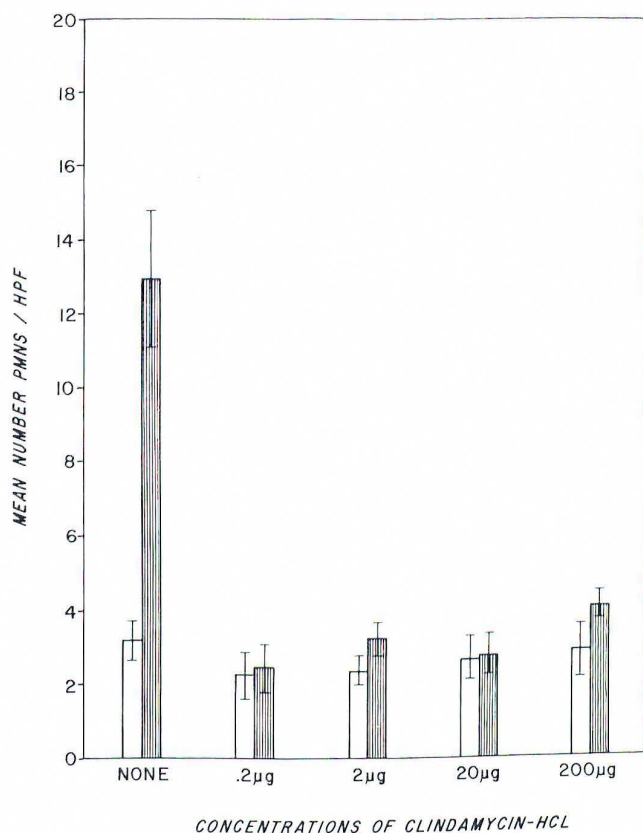


FIG 3. Chemotactic activity of human leukocytes with different concentrations of *clindamycin HCl* added to the lower compartment of the chamber. Clear bars represent the mean of 8 assays (± 1 SD) for random migration of leukocytes. Dark bars represent mean values (± 1 SD) for neutrophil chemotaxis.

In the second set of experiments when the drugs were added directly to the leukocyte suspensions prior to incubation, the results obtained were comparable to those depicted in Fig 1-5 (Table). Tetracycline HCl, erythromycin base and clindamycin-HCl at the same concentrations indicated in the figures caused marked suppression of chemotaxis and minimal suppression of random migration. The mean values for random migration fell slightly below the control values, and were the same order of magnitude as in the first set of experiments. The only significant difference noted between this set of experiments and those described above was a failure of clindamycin HCl to inhibit chemotaxis when added in a concentration of 200 $\mu\text{g/ml}$ ($p < .0005$). Penicillin G-Na, sulfapyridine and dapsone had no effect on leukocyte chemotaxis when added to the upper compartment of the chamber.

DISCUSSION

Antimicrobial agents are frequently used to treat inflammatory disorders of the skin that are characterized by an influx of polymorphonuclear leukocytes but are not due to bacterial infection. Although several hypotheses have been advanced to account for the effect of these drugs in inflammatory conditions, the proposed mechanisms of action are still controversial, owing to conflicting data. A possible effect on leukocyte migration has received little consideration in the development of these concepts; therefore, it seemed of interest to study the interaction of antimicrobial drugs and polymorphonuclear leukocytes in an assay system for chemotaxis.

The modified micropore chamber has proved to be a reliable system in which the chemotactic response of white blood cells can be assayed in the laboratory [21]. This technique has

been utilized in previous studies to demonstrate the effect of drugs on the migration of polymorphonuclear leukocytes. The antiinflammatory agents, chloroquine, hydrocortisone, and methyl prednisolone have been shown to suppress chemotaxis by direct action on the neutrophil [22]. Mafenide acetate and sulfadiazine compounds, widely used for the treatment of burns, also appear to cause marked inhibition of leukocyte motility [19].

Martin et al. observed reduced migration of leukocytes obtained from human volunteers with *Mycoplasma pneumoniae* infection who were receiving tetracycline [23]. Subsequent studies demonstrated that concentrations of tetracycline ranging from 0.01 to 10 $\mu\text{g/ml}$ depressed both random migration and chemotaxis of leukocytes toward *Mycoplasma* antigen [14]. In contrast, concentrations of tetracycline ranging from 30-300 $\mu\text{g/ml}$ caused stimulation of neutrophil movement. The mechanisms by which the drug influences white cell movement are unknown, but it has been suggested that these effects may relate to alterations in white cell metabolism.

Although we used zymosan-induced chemotactic factor rather than *Mycoplasma* antigen as a chemoattractant, our studies confirm the findings of Martin and co-workers. Concentrations of tetracycline in the range of therapeutic blood levels (0.1-10 $\mu\text{g/ml}$) appeared to inhibit neutrophil chemotaxis whereas tetracycline in very high concentration (300 $\mu\text{g/ml}$) failed to suppress chemotaxis. The effect of tetracycline on random migration was less pronounced in our assay system than in the study of Martin et al. This disparity in results may be attributable to several differences in methodology. It is unclear whether the tetracycline was added to the upper

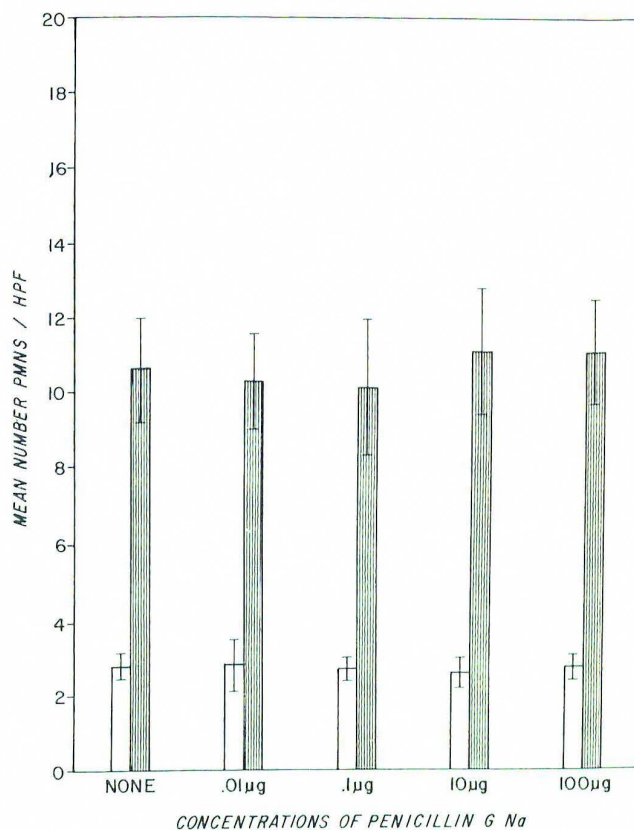


FIG 4. Chemotactic activity of human leukocytes with different concentrations of *penicillin G-Na* added to the lower compartment of the chamber. Clear bars represent the mean of 8 assays (± 1 SD) for random migration of leukocytes. Dark bars represent mean values (± 1 SD) for neutrophil chemotaxis.

Chemotactic activity of human leukocytes with different concentrations of antimicrobial agents added to the upper compartment of the chamber. Values are expressed as means of 8 assays ± 1 SD

Tetracycline		Erythromycin		Clindamycin		Penicillin		Sulfapyridine		Dapsone	
None	2.5 \pm .5	None	3.0 \pm .3	None	2.2 \pm .4	None	2.4 \pm .1	None	3.7 \pm .5	None	3.7 \pm .5
Zymosan	12.7 \pm .7	Zymosan	11.2 \pm .3	Zymosan	11.0 \pm 1.0	Zymosan	13.3 \pm .5	Zymosan	13.5 \pm .9	Zymosan	13.5 \pm .9
.01 μ g	2.2 \pm .3	.01 μ g	2.5 \pm .8	.2 μ g	2.0 \pm .1	.01 μ g	2.1 \pm .1	100 μ g	3.6 \pm .4	50 μ g	3.2 \pm .4
.01 μ g + Z ^a	2.2 \pm .5	.01 μ g + Z	2.2 \pm .5	.2 μ g + Z	2.2 \pm .2	.01 μ g + Z	13.1 \pm .8	100 μ g + Z	14.1 \pm .7	50 μ g + Z	13.7 \pm 1.5
1 μ g	1.9 \pm .4	1 μ g	2.3 \pm .2	2 μ g	2.2 \pm .1	.1 μ g	2.1 \pm .2	50 μ g	3.7 \pm .9	25 μ g	3.1 \pm .2
1 μ g + Z	2.4 \pm .2	1 μ g + Z	2.2 \pm .1	2 μ g + Z	2.1 \pm .2	.1 μ g + Z	12.5 \pm .8	50 μ g + Z	14.4 \pm 1.8	25 μ g + Z	13.6 \pm 1.3
10 μ g	2.0 \pm .4	10 μ g	2.4 \pm .2	20 μ g	2.2 \pm .2	10 μ g	2.1 \pm .2				
10 μ g + Z	2.6 \pm .4	10 μ g + Z	2.2 \pm .3	20 μ g + Z	2.6 \pm .6	10 μ g + Z	13.2 \pm 1.6				
300 μ g	2.9 \pm .4	300 μ g	2.3 \pm .4	200 μ g	2.2 \pm .3	100 μ g	2.3 \pm .2				
300 μ g + Z	12.7 \pm 1.7	300 μ g + Z	12.0 \pm .8	200 μ g + Z	10.8 \pm .9	100 μ g + Z	13.7 \pm .6				

^a Z = zymosan

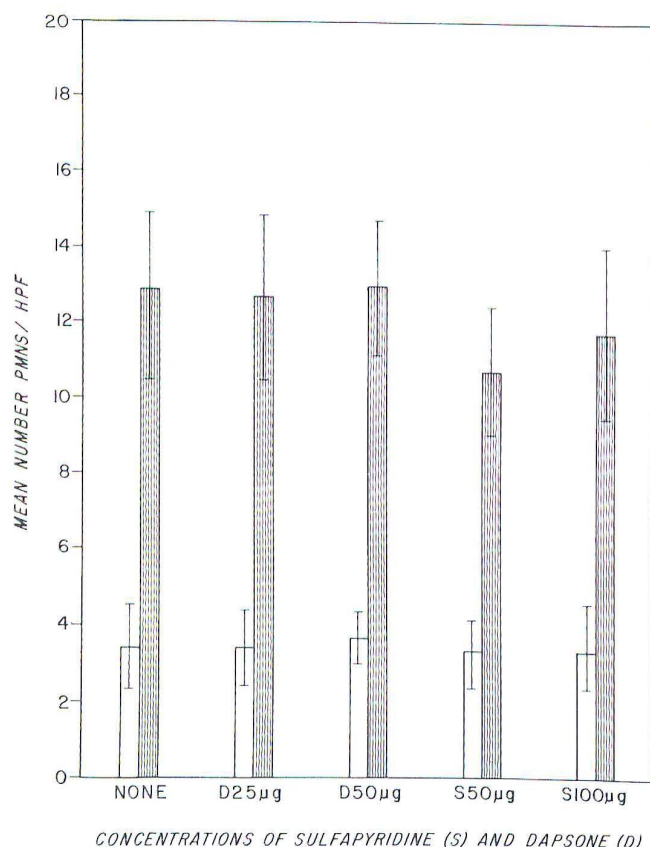


FIG 5. Chemotactic activity of human leukocytes with different concentrations of sulfapyridine and dapsone added to the lower compartment of the chamber. Clear bars represent the mean of 8 assays (± 1 SD) for random migration of leukocytes. Dark bars represent mean values (± 1 SD) for neutrophil chemotaxis.

(leukocyte) compartment or the lower (chemoattractant) compartment of the chamber or whether the leukocytes were preincubated with tetracycline prior to assay in the study of Martin et al. Other investigators [22,24] have shown that suppression of white cell movement is more pronounced when the inhibiting substance is added to the leukocyte rather than the chemoattractant side of the micropore filter. In addition, preincubation of leukocytes with drug prior to injection into the chamber may augment the effects on leukocyte motility [24]. Possibly our failure to demonstrate a dramatic

effect on random migration may reside in this latter procedural difference.

Erythromycin base and clindamycin HCl, like tetracycline, also appear to inhibit leukocyte motility in vitro. These data support the findings of Plewig and Schöpf [15] in a study of the effects of antimicrobial agents on pustular dermatitis in humans, induced by 40% potassium iodide ointment. Topical application of tetracycline and erythromycin suppressed the pustular reaction whereas penicillin G-Na and diaminodiphenylsulfone had no effect. Oral administration of tetracycline and erythromycin also inhibited the pustular reaction to topical potassium iodide, but oral penicillin G-Na failed to suppress the dermatitis. Systemically administered diaminodiphenylsulfone exhibited the most pronounced antiinflammatory effect in contrast to its ineffectiveness when applied topically. The concentrations of diaminodiphenylsulfone used in our study are higher than the blood levels that would be expected from the oral dosages utilized by Plewig and Schöpf [25-27]. Although we detected no suppression of leukocyte chemotaxis, it is conceivable that suppression might be observed at lower concentrations. Alternatively, the effect of diaminodiphenylsulfone on the inflammatory response may be mediated through mechanisms other than chemotaxis [27-30], however, these data are controversial [31].

Plewig and Schöpf postulated that their data might be explained by an effect of specific drugs on leukocyte migration. Our data support this hypothesis and suggest that inhibition of leukocyte movement may, in some circumstances, be an important mechanism by which certain antimicrobial agents suppress inflammatory skin disease.

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