STUDIES ON HUMAN LEUKOCYTE MOTILITY

I. Effects of Alterations in pH, Electrolyte Concentration, and Phagocytosis on Leukocyte Migration, Adhesiveness, and Aggregation*

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Multiple factors influence the initial inflammatory response to microbial lodgement in tissues. These factors include the nature of the invading microorganisms, vascular and lymphatic events associated with their presence, and the speed and intensity of polymorphonuclear leukocyte migration to the site of infection. In recent years, considerable attention has been focused on the late inflammatory events of phagocytosis and the subsequent fate of ingested microorganisms. Because factors affecting leukocyte motion appeared less well characterized, the present studies were undertaken utilizing a simple in vitro system which permitted quantitative measurements of leukocyte migration under a variety of circumstances. This paper reports the effects of changes in pH, electrolyte concentration, plasma factors, and phagocytosis on the ability of human polymorphonuclear leukocytes to migrate against gravity in glass capillary tubes.

Methods

Leukocyte migration was measured in vertically positioned microhematocrit tubes using a modification of the method described by Ketchel and Favour (1). Venous blood was obtained from normal human donors and anticoagulated with heparin to a final concentration of 4 mg/100 ml. Siliconized 32 mm Drummond capillary tubes stored in 95% ethyl alcohol were flame-dried prior to use, filled to 70% capacity with test blood, sealed by heat, and centrifuged for 2 min in a Drummond microhematocrit centrifuge. The tubes were then arranged side by side and fixed with paraffin on a glass slide which was mounted on a vertically arranged microscope stage as shown in Fig. 1.

Cell migration from the point of origin to the advancing front of migrating cells was measured with an ocular micrometer. The ten most advanced cells were excluded to obtain a more compact border for measurements. Seven to ten tubes were employed for each variable tested, and measurements of cell migration were recorded at 1, 2, and 4 hr. Studies were carried out

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at 37°C except in experiments testing the effects of temperature. Statistical significance of measurements was determined by *t* test analysis. In studies in which individual leukocyte velocity was determined, an oculometer at 970 × magnification was employed to permit measurement of total linear cell motion over a 5 min period.

RESULTS

Normal Leukocyte Migration.—Fig. 2 illustrates the dynamics of leukocyte migration observed in 89 determinations performed on 5 consecutive days using cells and plasma from one individual. Deviation from mean values on the same

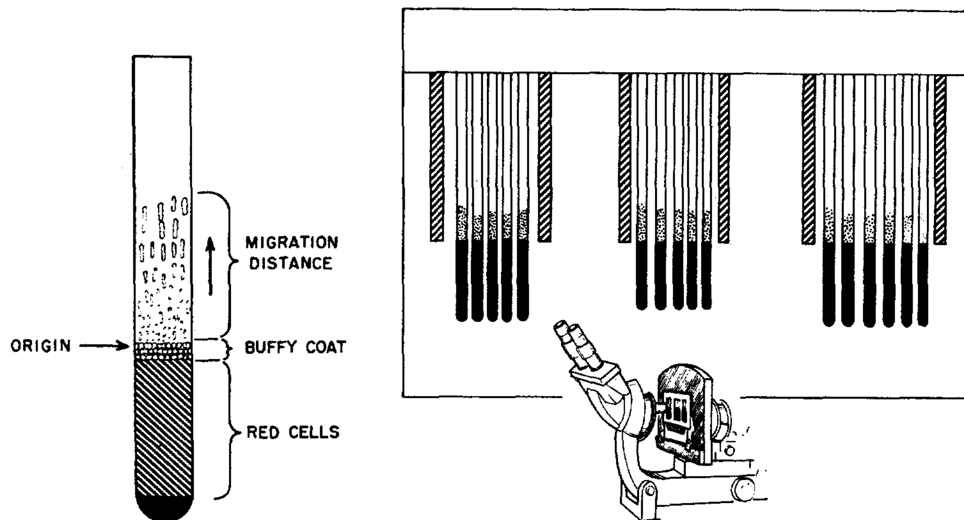

FIG. 1. The technique used for study of leukocyte migration. After centrifugation, tubes were mounted on a glass slide and observed at 100 × magnification. Migration distance was measured with an oculometer.

day rarely exceeded ±0.24 mm. Although migration continued for 12 hr, the first 4-hr period was selected for detailed observation because of its consistency and reproducibility. As seen at 100 × magnification, the leukocyte population moved vertically up the tube at a decreasing rate with time. That the apparent change in cell migration rate was due to the increasing opportunity for leukocytes at the advancing border to move randomly in any direction, rather than to decreasing cell motility per se, was shown by studies at higher magnification. When the rates of migration of the individual cells making up the advancing border were measured at 970 × magnification, individual cell locomotion rates did not decrease significantly over the 4-hr period. That this system was sufficiently sensitive to detect small influences on cell motility was shown by

studies employing changes in temperature and in sodium arsenite concentration.

Modification of Leukocyte Migration with Temperature.—Leukocyte migration increased with increasing temperature over the range studied (25° to 40°C) (Fig. 3). With each degree of increase in temperature, migration rates increased

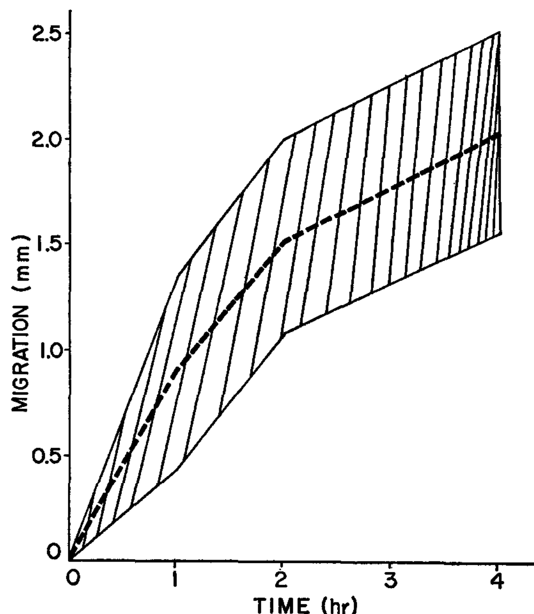

FIG. 2. Normal human leukocyte migration in capillary tubes. Mean cumulative leukocyte migration distance is indicated by dashed line. Two standard deviations from the mean are represented by shaded areas.

by approximately 10%. Migrations at 37°C and 40°C were significantly different ($P<0.05$). Migration at 42°C was slightly less than that observed at 40°C and and was not included in Fig. 3.

The Effect of Arsenite on Leukocyte Migration.—

One part sodium arsenite in various concentrations was added to 19 parts blood to provide the final arsenite concentrations shown in Fig. 4. Appropriate controls were prepared using distilled water in place of the arsenite solution. Specimens were incubated at 37°C for 30 min prior to testing.

Cell motion was unaffected by 0.01 mM sodium arsenite, but was progressively retarded in higher arsenite concentrations. Inhibition of migration was complete in 0.25 mM sodium arsenite (Fig. 4). The difference in migration in 0.01

and 0.05 mM sodium arsenite concentrations was statistically significant ($P < 0.005$).

The Effect of Hyperglycemia and Hypoglycemia on Leukocyte Migration.—

Hyperglycemia was simulated by adding appropriately diluted dextrose solution to produce the plasma glucose levels indicated in Fig. 5. Glucose concentrations were determined by the method of Hoffman (2). Hypoglycemia was simulated by diluting 1 ml of blood with 10 ml of normal saline containing 3 meq/liter of potassium chloride. The mixture was then centrifuged and 10 ml of the supernatant was removed, restoring a normal hematocrit. 0.15 ml of plasma, dialyzed to reduce glucose concentration to less than 5 mg/100 ml, was then added to each 1.0 ml of the cells-in-saline mixture; this provided a concentration of plasma shown to be adequate to sustain optimum migration. The control system was identically prepared with a glucose concentration of 100 mg/100 ml.

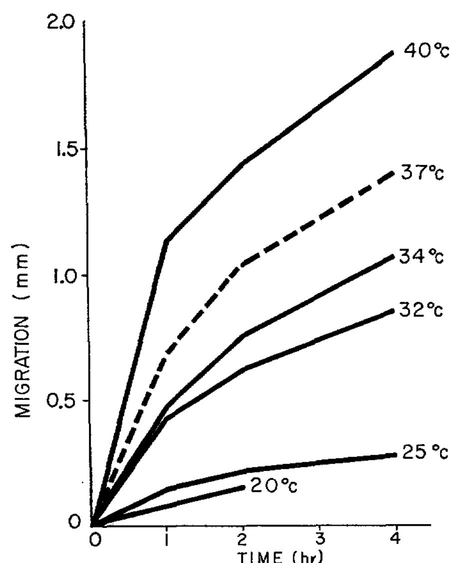

FIG. 3. Effect of temperature on leukocyte migration.

As shown in Fig. 5, leukocyte motility was unaffected by variations in glucose concentration ranging from 1240 to 50 mg/100 ml. Migration in plasma containing 10 mg/100 ml glucose was slightly retarded, but reduction in plasma glucose to 6 mg/100 ml or below was required before migration was significantly reduced ($P < 0.005$).

The Effect of Acidosis on Leukocyte Migration.—

Metabolic acidosis was simulated by adding blood to a 50 per cent dilution of plasma in Hanks' solution adjusted to the desired pH with lactic acid, beta hydroxybutryic acid, or

hydrochloric acid.0.5 ml aliquots of blood were incubated with 3.0 ml aliquots of variously modified Hanks' solutions for 1 hr. Specimens were centrifuged, 3.0 ml of supernatant removed to restore a normal hematocrit, and migration studies then performed. pH of the supernatant was determined immediately after incubation. Control specimens were similarly treated using Hanks' solution at pH 7.6. *Diabetic acidosis* was simulated by adding 0.5 ml heparinized blood to 3.0 ml of Hanks' solution containing 50 mg/100 ml acetone, 50 mg/100 ml beta hydroxybutyric acid, or 50 mg/100 ml beta hydroxybutyric acid in 1000 mg/100 ml glucose. Incubation was carried out for 1 hr, the mixture centrifuged, and 3.0 ml of supernatant then removed. The sedimented cells were shaken, 0.15 ml plasma was added to 0.5 ml of cell suspension, and migration studies were performed. *Respiratory acidosis* was simulated by bubbling 30% carbon dioxide in oxygen through 1.0 ml of heparinized blood diluted with 3.0 ml of plasma. The added plasma was then removed and pH determined immediately.

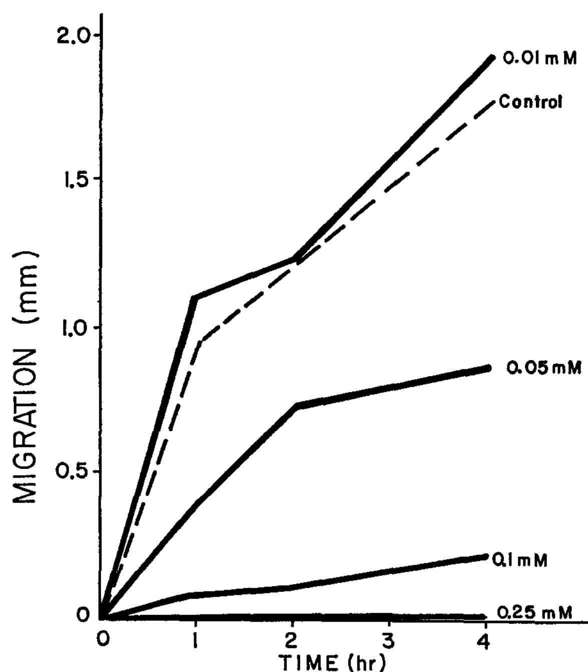

FIG. 4. Effect of sodium arsenite at various concentrations on leukocyte migration.

As shown in Fig. 6, leukocyte migration was unmodified by marked reductions in pH produced by additional hydrochloric acid, lactic acid, or beta hydroxybutyric acid. Similarly, no changes in leukocyte migration could be demonstrated in systems altered to simulate respiratory acidosis.

The Effect of Serum Electrolytes on Leukocyte Migration.—

Hypercalcemia and hyperkalemia were produced by adding calcium gluconate or potassium chloride to heparinized blood. Deficiencies in these ions were produced by the use of plasma

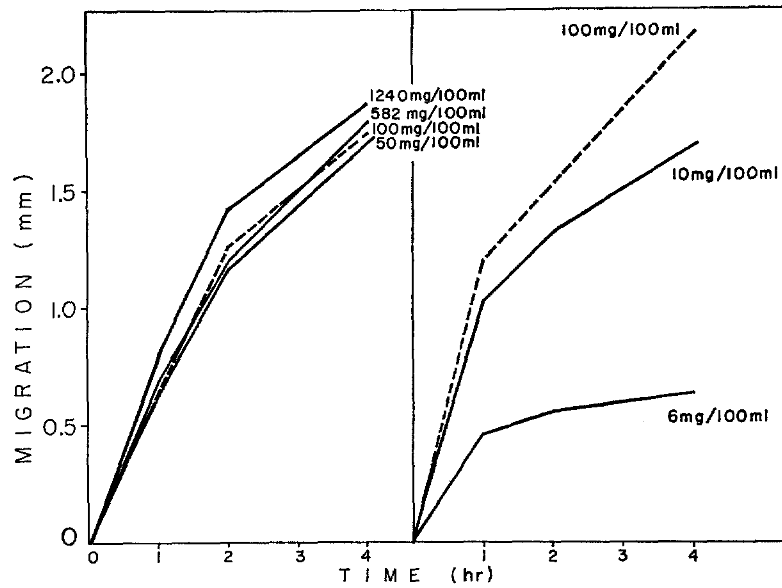

FIG. 5. Leukocyte migration in plasma containing various concentrations of glucose. The dashed lines represent the control system.

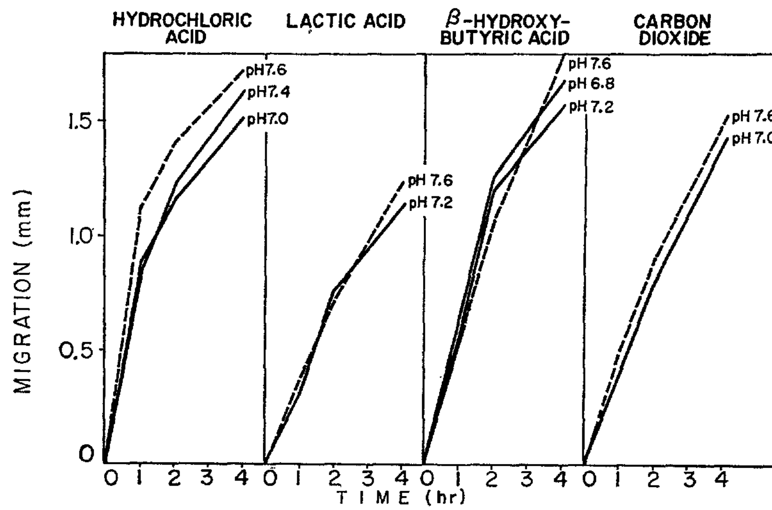

FIG. 6. Effect of acidosis on leukocyte migration. Studies with each agent were performed on different days and on blood from different subjects. The dashed lines represent control studies run simultaneously.

dialyzed against saline at 20°C. Hypernatremia and hyponatremia were produced by adding hypertonic saline or distilled water to heparinized blood. Isotonic saline additives were used in control experiments.

Studies utilizing various salts of EDTA permitted analysis of the role of calcium ion and magnesium ion in the phenomena under study. Na_2H_2EDTA (NaEDTA) binds both calcium and magnesium virtually completely (dissociation constants 10^{-11} and 10^{-8} respectively); $Na_2MgEDTA$ (MgEDTA) binds calcium to the same degree but does not bind magnesium; $Na_2CaEDTA$ (CaEDTA) binds neither ion and therefore served as a control for the effects of the EDTA molecule per se.

Two further maneuvers were employed to distinguish changes in the property of adherence to glass from changes in intrinsic cell motion. Capillary tubes were positioned on their sides for 1 hr to allow lateral sedimentation of cells, and then centrifuged in a vertical position for 10 sec to sediment erythrocytes. This maneuver allowed leukocytes to adhere to the capillary walls and removed erythrocytes from the areas being inspected. Leukocytes adherent to capillary tube walls after this procedure were counted as an index of the degree of adherence to glass. Individual cell movement was determined by measuring total linear motion of single cells observed on a horizontally placed cover slip for 5 min periods.

Sodium concentrations ranging from a low of 120 meq/liter to a high of 164 meq/liter did not alter leukocyte migration. Similarly, changes in chloride concentrations from 84 to 143 meq/liter did not produce detectable effects. In like manner, leukocyte migration was unchanged by wide variations in calcium and potassium concentrations (Fig. 7).

NaEDTA in concentrations exceeding the sum of calcium and magnesium ion concentrations completely eliminated leukocyte migration in capillary tubes (Fig. 8). Further studies showed that this effect appeared to be due to the inability of leukocytes to adhere to glass (Table I). That such adhesion was dependent on the presence of Mg^{++} rather than Ca^{++} could be shown by the fact that migration and intrinsic cell motility were unimpaired when MgEDTA was substituted for NaEDTA. CaEDTA did not affect leukocyte migration.

Heat-Labile Plasma Factors Affecting Leukocyte Migration.—

Cell-free plasma was heated to 56°C for 10 min or incubated with 0.08 M hydrazine. 0.14 ml of heat-inactivated or hydrazine-treated plasma was added to 0.5 ml washed cells and migration studies were performed. Controls utilized untreated plasma in identical fashion. A mixture of one-half heat-inactivated and one-half hydrazine-treated plasma was also tested in the same fashion for the ability to sustain leukocyte migration and to function as a complement source in C′-dependent hemolytic systems. The effect of these altered plasmas on the property of leukocytic adhesion to glass was also determined as described previously.

As shown in Fig. 9, neither heated plasma nor hydrazine-treated plasma permitted normal migration of washed cells. A 50% mixture of heated plasma and hydrazine-treated plasma sustained normal complement-dependent hemolysis but did not restore migration. Leukocyte adhesiveness to glass was significantly reduced in such heated plasma (Table II). Leukocyte motility in horizontally placed cover slip preparations was also reduced. Thus it was demonstrated that

the plasma component required for migration was heat labile, was inactivated by hydrazine, was not identical with hemolytic complement, and partially effected the adhesion of leukocytes to glass.

The Effect of Phagocytosis on Leukocyte Migration.—

Appropriately diluted viable or heat-killed staphylococci from 18 hr broth cultures, or latex particles having a mean size of 0.81 mμ (Difco Corp., Detroit), were placed in 1 ml of heparinized blood in stoppered, siliconized, sterile test tubes. These were rotated for 20 min at 37°C and further incubated without rotation for 20 min before migration studies were carried out. Cells and plasma from this system were subsequently observed for ability to migrate or sustain migration. The ratio of bacteria to cells was determined by standard pour plate methods, and the ratio of latex particles to cells by direct enumeration in Petroff-Hausser chambers.

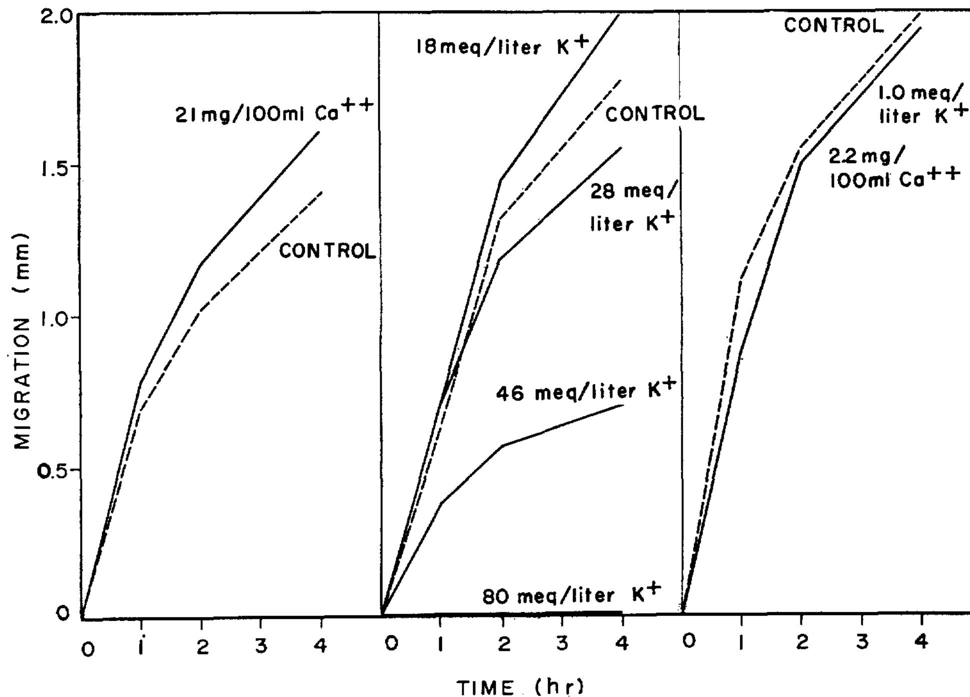

FIG. 7. Leukocyte migration in plasma containing various concentrations of calcium and potassium.

The effect of phagocytosis on leukocyte migration is illustrated in Fig. 10. Migration was significantly reduced after phagocytosis of viable coagulase-positive staphylococci, heat-killed staphylococci, or coagulase-negative staphylococci. Ingestion of latex particles had a similar effect, but a higher particle-

leukocyte ratio was necessary to achieve equivalent inhibition. Reduction in migration appeared to relate to the phagocytic process and correlated in degree with the number of particles available for ingestion.

This reduction in migration after phagocytosis appeared to be a consequence

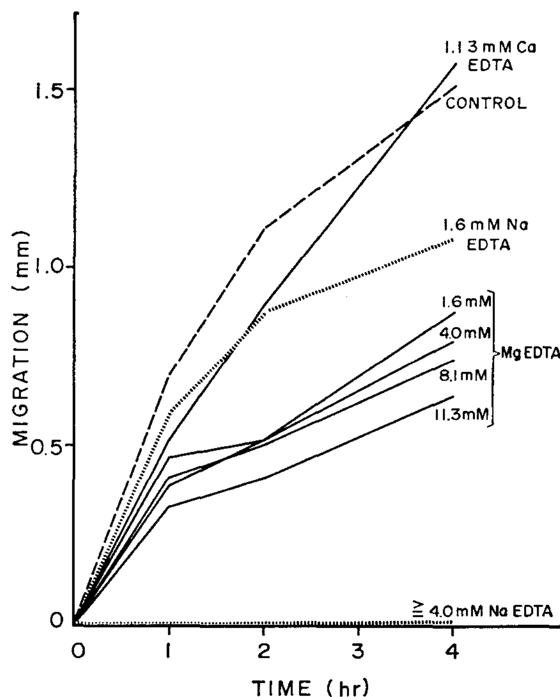

FIG. 8. Effects of various salts of EDTA on leukocyte migration. The dashed line represents the control study, the dotted lines represent studies using NaEDTA, and the solid lines represent studies using MgEDTA or CaEDTA as indicated.

TABLE I

Effect of Na_2H_2EDTA and $Na_2MgEDTA$ on Leukocyte Adhesion to Glass

Chelating agent	No. of cells adhering to capillary tube walls*
None (saline)	669 ± 61
1.6 mM $Na_2MgEDTA$	706 ± 105
12.0 mM $Na_2MgEDTA$	1301 ± 99
1.6 mM Na_2H_2EDTA	583 ± 53
12.0 mM Na_2H_2EDTA	0.6 ± 0.3

* Mean ± standard error of mean.

of cellular change rather than alteration in plasma. When fresh leukocytes were placed in plasma removed from such phagocytic systems, normal migration occurred. Conversely, fresh plasma did not restore normal migration to cells previously participating in phagocytosis.

The effect of phagocytosis in reducing leukocyte migration as observed in

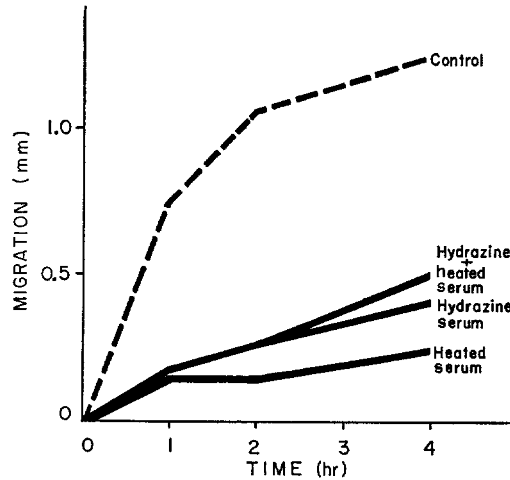

FIG. 9. Effect of heated plasma and hydrazine-treated plasma on leukocyte migration.

TABLE II

Effect of Heated Plasma on Leukocyte Adhesion to Glass

Plasma added to washed cells	No. of cells adhering to capillary walls*
Heated plasma (56°C for 30 min)	112 ± 20
Fresh plasma	894 ± 40

* Mean ± standard error of mean.

capillary tubes was shown to be complex, involving both aggregation of cells and decrease in intrinsic motility of individual leukocytes. Elaboration of leukotoxic substances secondary to phagocytosis could not be demonstrated. The following experiments document these statements.

1. *Leukocyte aggregation during phagocytosis:* Aliquots of blood containing various concentrations of bacteria were placed in paired capillary tubes, rotated at 37°C, and centrifuged in the usual fashion for migration experiments. One of each pair was observed for migration in the usual way, and the other was inverted for 1 hr to allow the buffy coat to be redistributed in plasma. At the end of this period, inverted capillary tubes were placed flat in the bottom of large centrifuge cups and centrifuged at 2400 *g* for 2 min. This maneuver produced maximum adherence of the leukocytes to the capillary wall. Finally, erythrocytes were resedimented by

10 sec conventional centrifugation in the Drummond microhematocrit centrifuge. Aggregated and nonaggregated leukocytes were then counted at 100 × magnification. The proportion of polymorphonuclear leukocytes containing bacteria (PCB) was determined using cover slip preparations from each blood sample. Controls consisted of similarly treated blood specimens without bacteria.

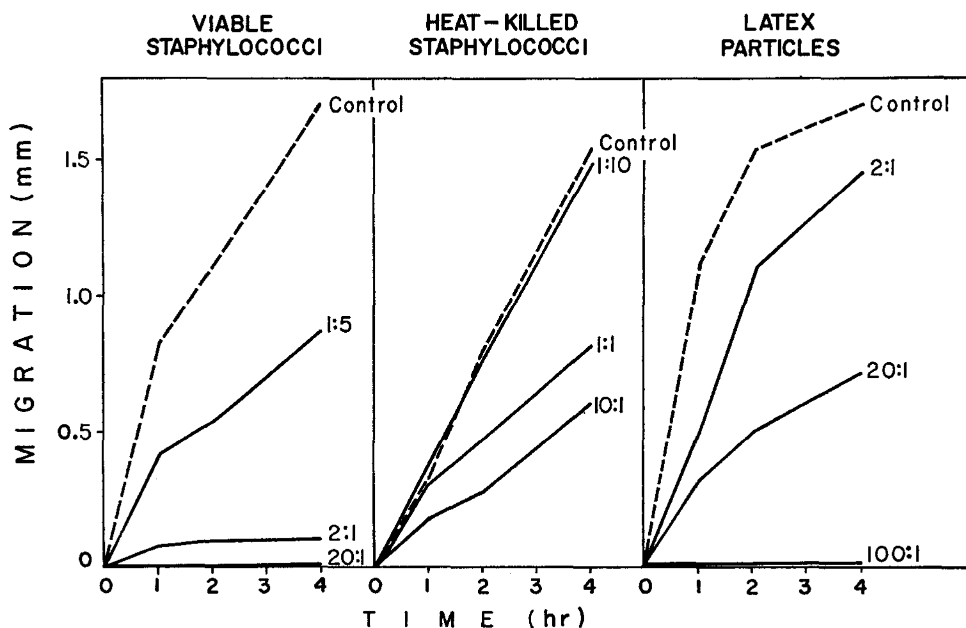

FIG. 10. Effects of phagocytosis on leukocyte migration. Left, impaired migration after ingestion of viable staphylococci; center, effect of heat-killed staphylococci; right, effect of latex particles. Ratios refer to the number of bacteria or latex particles per leukocyte.

Fig. 11 shows that leukocyte migration was reduced and leukocyte aggregation increased as the percentage of leukocytes containing bacteria increased.

2. *Impairment of individual cell motility by phagocytosis:*

a) Capillary tubes were prepared in the usual fashion using blood inoculated with bacteria or latex balls in which phagocytosis had been permitted, as previously described. By the use of high (970 ×) magnification, individual nonaggregated polymorphonuclear leukocytes (PMN) at the advancing cell margin could be observed and the number of bacteria contained within each cell enumerated. Over 300 observations were made in which the total linear distance traversed in any direction by individual PMN's over a 5 min period was determined using the ocular micrometer scale.

b) In parallel studies, blood samples from the same phagocytic experiments were used for study of vertical cell migration in the usual fashion, horizontal migration on cover slip preparations, and cell appearance and phagocytic indices in preparations stained with Wright's stain. At the end of the capillary tube migration studies, cells adherent to capillary tube walls

were fixed with methanol and then stained with Wright's stain. The number of bacteria per PMN and the type of leukocyte present at various levels of the migrating column were determined by direct microscopy.

Fig. 12 shows that the reduction in both total migration and individual PMN motility bore a direct relation to the number of bacteria contained in individual leukocytes. The number of bacteria contained in leukocytes at the advancing cell margin was significantly lower than the average numbers contained in PMN's in cover slip preparations. In tubes in which less than 100% of the leukocytes contained bacteria, the majority of the leukocytes at the advancing cell margin did not contain microorganisms.

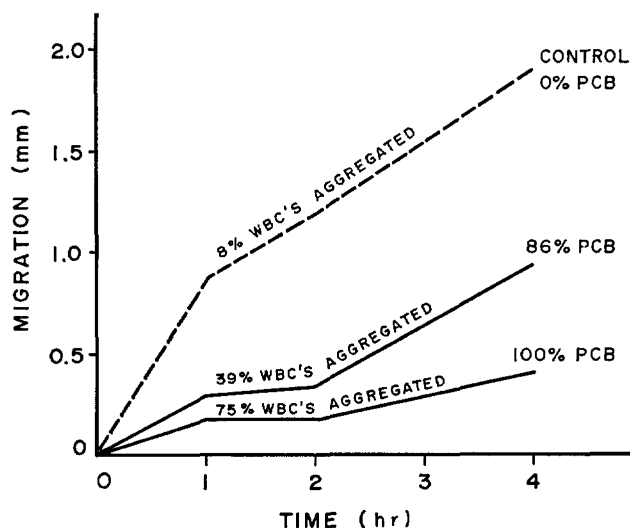

FIG. 11. Effect of bacterial ingestion on leukocyte migration and aggregation. The mean number of bacteria within polymorphonuclear leukocytes (PMN) was calculated from observations on 100 PMN's in each system. PCB = PMN containing bacteria.

3. *Studies on possible liberation of leukocyte-modifying substances of small molecular size during phagocytosis:* A 3.0 ml heparinized blood sample containing 3.0×10^8 heat-killed bacteria was placed in a sealed cellulose tubing dialysis bag (Fisher) and immersed in 3.0 ml heparinized bacteria-free blood in a sterile stoppered siliconized test tube. Incubation and rotation were carried out as usual. Migration studies were then performed using the blood inside the dialysis bag (PMN's containing bacteria) and the blood surrounding the dialysis bag (PMN's subjected to dialyzable substances released from the bacteria-leukocyte mixture). Control speciments containing no bacteria were treated in identical fashion.

As shown in Fig. 13, no evidence could be obtained to suggest that the factor causing impaired migration and leukocyte agglutination in phagocytic systems passed through a cellulose dialysis membrane.

DISCUSSION

Though polymorphonuclear leukocyte motility has been studied by many methods, most have not distinguished the contributions of individual cell motility, the effects of leukocyte aggregation, and the ability of cells to adhere

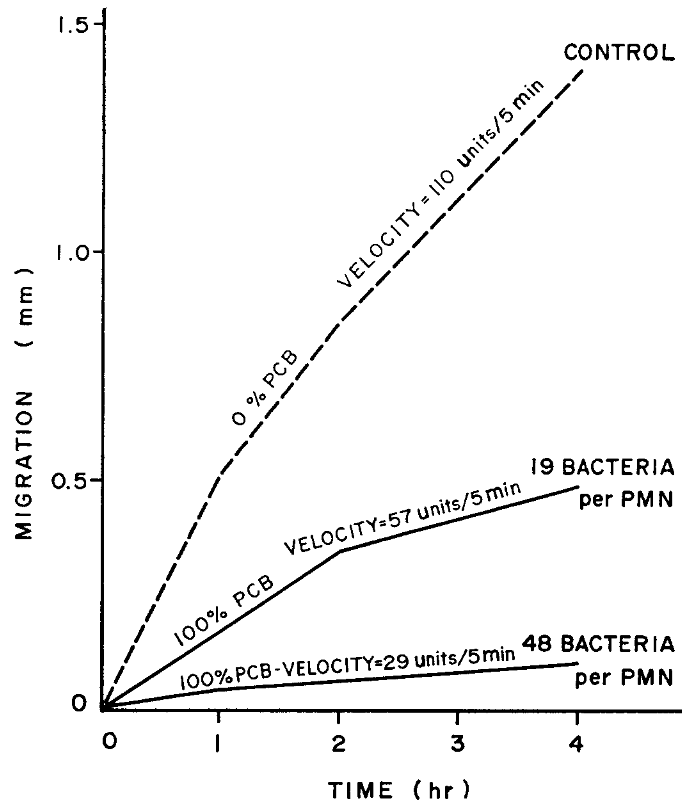

FIG. 12. Effect of bacterial ingestion on individual PMN motility. Reduction in velocity after phagocytosis is directly proportional to the number of bacteria contained in the leukocyte ($P < 0.001$). PCB = PMN containing bacteria.

to glass to the over-all effects observed (3–7). The present method permitted evaluation of all these features. Normal leukocytes in plasma systems migrated in a uniform and reproducible manner. Individual cell motility could be observed, adhesion to glass quantitated, and specimens could be subsequently fixed, stained, and viewed *in situ* at high magnification to quantitate the degree of leukocyte aggregation and particle ingestion.

The present studies are interpreted as indicating the following:

1. Leukocyte migration in capillary tubes depends both on cell motility and on leukocyte adhesion to glass.

2. Normal migration requires the presence of certain factors present in serum or plasma, and of magnesium ion. Leukocyte migration is reduced in plasma heated to 56°C for 10 min or plasma treated with hydrazine. This reduction in motility is due in part to reduced cellular adhesion to glass in these plasmas. The plasma factor or factors involved do not appear to be complement. Similarly, cellular adhesion to glass

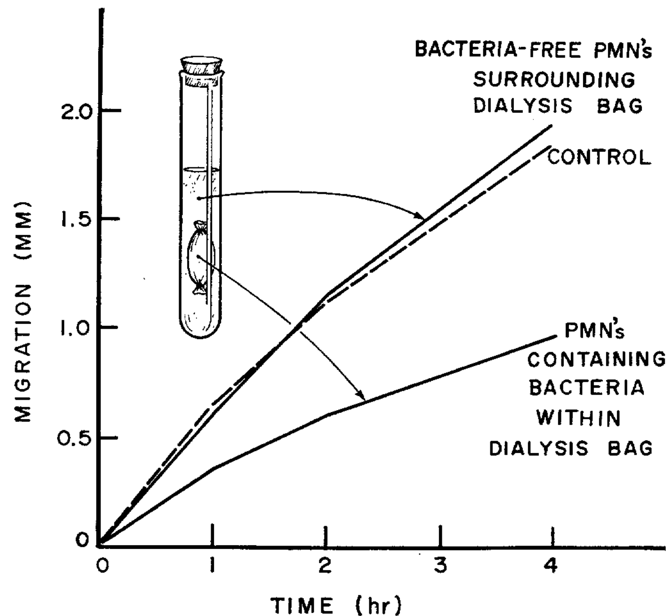

FIG. 13. Effect of phagocytosis on the motility of PMN's separated from bacteria by a dialysis membrane. No reduction in motility was observed in cells outside the membrane.

requires the presence of magnesium ion, but is not impaired in the virtual absence of Ca^{++}.

3. Leukocyte migration is resistant to alterations in the plasma milieu, including marked acidosis or wide variations in sodium, potassium, or calcium concentrations.

4. Phagocytosis of particulate matter results in marked impairment of leukocyte migration. This reduction in motility is due both to decreased individual cell motility per se and to aggregation of leukocytes participating in the phagocytic process.

Serum or plasma factors necessary for leukocyte migration in glass capillary tubes were heat sensitive and hydrazine sensitive, but differed from complement as classically defined. Such factors thus appear to resemble the heat-labile non-complement opsonic substances which facilitate ingestion of microbes by leukocytes (8, 9). Ketchel and Favour using identical techniques described serum

motility factors which were destroyed by heating to 56°C, restored by addition of Cohn fraction II, and inhibited by addition of Cohn fraction III (1). In their studies no attempt was made to differentiate changes in intrinsic cell motility from changes in the ability of cells to adhere to and move along glass surfaces against gravity. The present observations indicate that the function of this serum factor or factors may relate more to cellular adherence to glass than to intrinsic cell motility; leukocyte adhesion to the sides of capillary tubes was reduced in heated serum, whereas individual cell motility observed on horizontal surfaces was only slightly impaired.

The present demonstration that leukocyte motility is unimpaired by wide variations in glucose, electrolyte, and hydrogen ion concentrations is in accord with previous studies of chemotaxis and phagocytosis (10, 11). Polymorphonuclear leukocytes appear remarkably resistant to changes in plasma milieu. It seems unlikely that abnormal plasma concentrations of these substances found in disease states interfere with leukocyte motility per se. These observations are in keeping with previous studies on polymorphonuclear leukocyte activity in plasma obtained during diabetic acidosis (12).

Reduced polymorphonuclear leukocyte migration following phagocytosis has been recorded only infrequently. Martin and his coworkers (5) and Allgower and Block (7) showed that ingestion of tubercle bacilli resulted in decreased leukocyte motility and cell aggregation. Martin and Chaudhuri (13) also reported that leukocyte migration was inhibited by purified products obtained from Gram-negative bacteria, but indicated that intact staphylococci or pneumococci were without effects. The system employed by these investigators would not favor the occurrence of phagocytosis, and the effects noted were not attributed to phagocytosis. In the present studies staphylococci living or dead and even latex particles inhibited leukocyte migration and caused aggregation. This discrepancy in results may be due to the fact that Gram-negative products found to inhibit migration by Martin and Chaudhuri can be presumed to contain smaller and more dispersible particles which could be ingested even in systems not designed to enhance cell-particle contact.

In the present experiments, inhibition of leukocyte migration in capillary tubes following phagocytosis was mediated by both cellular aggregation and decreased intrinsic cellular motility. Individual nonaggregated cells were observed to move at reduced rates following phagocytosis. Decrease in individual cell motility was directly proportional to the number of bacteria ingested. Cohn and Morse have demonstrated increased leukocyte oxygen consumption, glucose utilization, and phagocytic activity after ingestion of staphylococci (10). Similar effects on leukocyte metabolism have been described following ingestion of polystyrene, latex, and starch particles (14, 15). Since cell motility is decreased as gross indices of cellular metabolism are increased, phagocytosis may divert cellular energy stores from motility to the more important functions

of particle ingestion and destruction. Increasing cellular metabolism, decreasing motility, and cellular aggregation following phagocytosis may favor localization and concentration of polymorphonuclear leukocytes in areas of bacterial invasion.

SUMMARY

Leukocyte migration was studied in a microhematocrit system which permitted evaluation of the separate effects of leukocyte adhesiveness, intrinsic cell motility, and leukocyte clumping. Leukocyte adhesion to glass required the presence of magnesium ion, was totally independent of calcium ion, and was partially dependent on heat-labile plasma factors. Leukocyte migration was unimpaired by marked acidosis or by wide variations in sodium, potassium, or calcium concentrations. Marked impairment of individual leukocyte motility and increased leukocyte aggregation were observed after phagocytosis of particulate matter, suggesting a mechanism facilitating recruitment and retention of leukocytes at areas of microbial invasion.

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