THE ACCELERATION AND INHIBITION OF MIGRATION OF HUMAN LEUCOCYTES IN VITRO BY PLASMA PROTEIN FRACTIONS*

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In this paper we have focused our attention on one fundamental property of leucocytes which can be measured quantitatively, namely their ability to migrate by ameboid motion. This report is part of an integrated study on the functions of leucocytes which has interested this group for some time. A preliminary report (Ketchel and Favour, 1953 *a*) indicates that factors occur in the plasma which influence strongly the rate of leucocyte migration. The present paper illuminates to some degree the nature of these factors, and also describes a new method of measuring leucocyte migration. In other investigations, the method has been found to have considerable usefulness in the study of leucocyte physiology (Ketchel and Favour, 1953 *b*: O'Neill and Favour, 1955).

Methods

As shown diagrammatically in Fig. 1, this technique utilizes a micro-hematocrit, formed inside a capillary tube of the type ordinarily used for melting point determinations. The tube is partially filled by capillarity when one end is held in a blood cell suspension. After the other end has been closed, the tube is centrifuged, resolving the suspension into three components; a basal layer of packed erythrocytes, an intermediate layer of leucocytes, and an uppermost layer of plasma. Upon incubation of the tube, the leucocytes migrate by ameboid motion into the plasma layer. The distance of migration is then measured by means of an ocular micrometer. The method provides a three-variable system in which the cells, the plasma, and any antigen, hormone, etc., may be varied. Elaborate controls may be included with little additional work. This simplicity of experimentation enables a single worker, in a relatively short time, to set up enough tubes to permit application of statistical methods in the analysis of data.

Glassware.—Seven cm. lengths of capillary tubing with an internal diameter of 0.8 ± 0.1

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mm. were used. Since attempts to clean the capillary tubes by a variety of methods produced toxic effects on the cells, new tubes were used for each experiment with no pretreatment except sterilization.

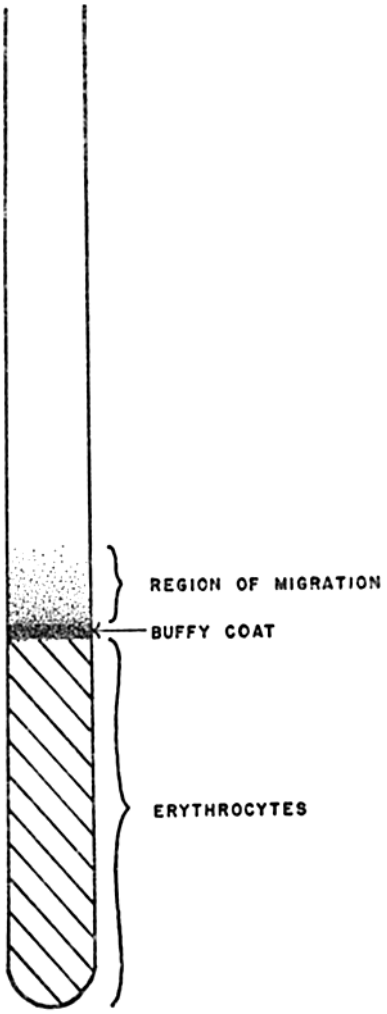

FIG. 1. Diagrammatic representation of the capillary tube method of measuring leucocyte migration.

All other glassware was coated with silicone,[1] cleaned thoroughly, and sterilized. Aseptic technique was used throughout the preparation of the cell suspensions and the loading of the tubes.

Cells.—Normal healthy subjects and hospitalized patients with various acute illnesses

[1] Dryfilm 9987, General Electric Company, Pittsfield, Mass.

served as donors. Ten ml. of venous blood was collected from each donor in syringes containing 0.5 ml. of a 2 mg./ml. heparin solution.[2] This concentration of heparin was chosen empirically as the amount which delayed clotting until the capillary tubes had been filled. Approximately 5 ml. of blood from each donor was discharged into individual 15 ml. centrifuge tubes and promptly centrifuged at 1200 *g* for 15 minutes. The clear supernatant plasma fractions were recentrifuged at 1600 *g* to clear them of platelets, and stored in an ice bath for later use in the experiment.

Each cellular fraction was then washed three times by successive resuspension and centrifugation in 4 volumes of the balanced salt solution of Hanks and Wallace (1949). While this part of the method had as its purpose the freeing of the cells of their autologous[3] plasma for experiments in which cells were tested in other plasmas, the procedure was carried out in all experiments to permit comparison of data. Storage of cells and subsequent preparation of cell suspensions were carried out in an ice bath.

Preparation of Capillary Tubes.—Following the washing of cells and the freeing of the plasma of formed elements, cells and plasma were recombined in the proportions indicated in the sample protocol (Table IV). The ingredients were adjusted so that the final plasma concentration was half the fluid volume, a plasma dilution which gave a clear, non-retractile clot in the capillary tube supernatant.

The microtubes were then loaded by capillarity to approximately ⅔ their length. The free end was promptly sealed in a high temperature flame, and cooled in alcohol.[4] Groups of tubes were centrifuged at 1600 *g* for 1 minute to form the three-phase system indicated in Fig. 1. Contact with the glass wall of the capillary tube by the cell suspension induced clotting within a minute to an hour or more. Tubes were inspected microscopically following centrifugation, and selected for absence of cells on the wall or in the plasma and for a sharp cell-plasma boundary. For each combination of cells and plasma 10 capillary tubes were used. With practice, it was possible for one person to prepare large numbers of capillary tube cultures within 3 to 4 hours of the initial venipuncture.

Cell Migration.—The capillary tubes were incubated vertically at 37°C. Within 2 hours, there appeared above the buffy coat a haze of discrete cells which, when watched individually, were seen to be actively migrating along the tube wall or in the plasma clot. These cells formed a zone with an easily discernible boundary. After 16 to 18 hours, the distance between the leading edge of this zone and the buffy coat was measured by means of an ocular micrometer. Repeat measurements on the same tube by different observers generally checked within 0.10 mm. A few cells were often seen in the plasma beyond the leading edge of the migrating zone, but since these cells seemed to be independent of the generalized migration from the buffy coat, they were ignored for the purposes of these experiments. The cells composing the moving boundary, as well as those with a faster rate of migration, were polynuclear cells.

To facilitate microscopy, groups of capillary tubes were secured at their upper ends to a 1 × 3 inch microscope slide with a drop of micromount[5] (Fig. 2). Fig. 3 shows the hourly migration of the leucocytes of two subjects with markedly differing migration rates. It can be seen that regardless of the migration rate, the migration of the leucocytes ceased at approximately the same time in both cell-plasma systems. Accordingly, the results in a given experi-

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[2] Liquaemin, Organon, Inc., Orange, New Jersey.

[3] Throughout the present paper the words "autologous" and "homologous" will have the usage usually employed by immunologists. Autologous will refer to cells and plasma from the same donor, and homologous will refer to cells and plasma from different donors.

[4] Cooling in water may fracture the capillary tubes.

[5] E. F. Mahady Company, Boston.

ment could be recorded either as the rate of migration in the first phase of the culture, or as the distance of migration after a given time. A single measurement at 16 to 18 hours was taken as a convenient end-point for the experiments in this study.

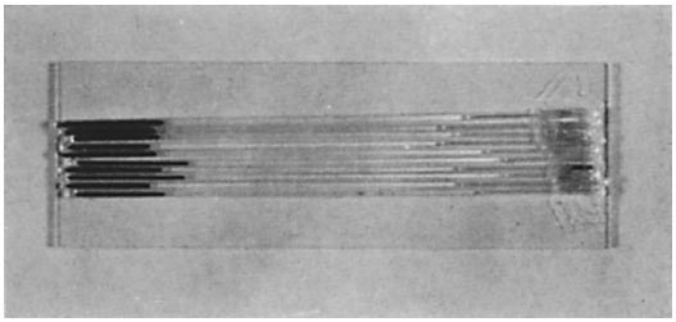

FIG. 2. A group of capillary tubes secured to a microscope slide to facilitate microscopy.

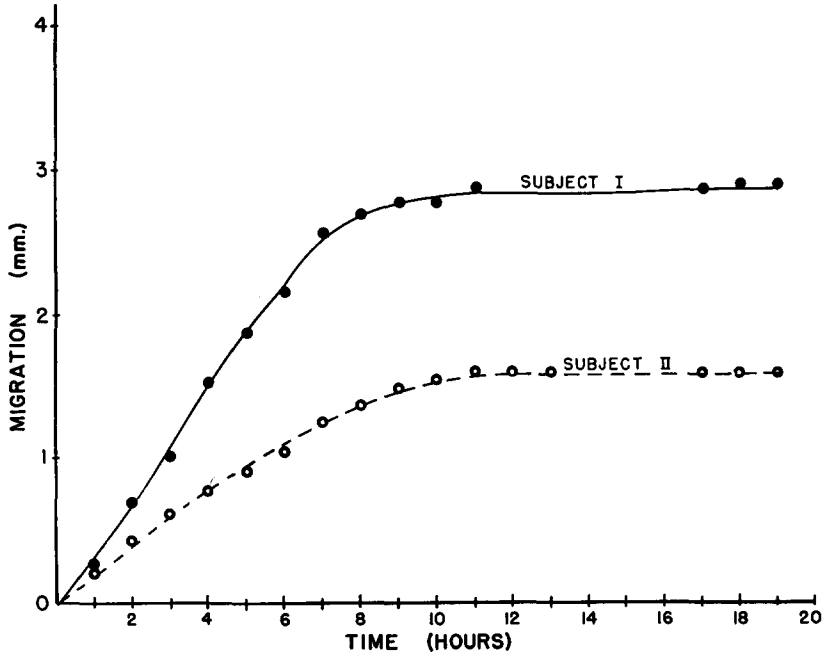

FIG. 3. Rate of leucocytes in their own plasmas, as observed in the cases of two different subjects. The difference in migration distance is the result of a difference in the rate of migration during the early hours of the experiment.

RESULTS

Individual Variation in Migration.—The first series of experiments (Table I) was performed with the blood of healthy laboratory personnel. All subjects

were studied repeatedly during a period of several weeks to several months, precautions being taken to avoid periods during which the individual showed symptoms of minor seasonal infections. The average distances of leucocyte migration of the 7 normal subjects ranged from 0.47 to 2.67 mm. As judged by the standard deviations (S.D.), the variations encountered in repeated tests on the blood of individual donors were not large for this type of measurement. It is apparent that, even though there is a great deal of variation in leucocyte migration from individual to individual, such normal healthy subjects show relatively little variation in their leucocyte migration distance from day to day.

Variations among Normal Individuals.—In an effort to determine the normal limits of leucocyte migration, a series of 78 normal individuals were each tested

TABLE I

Average Migration Distance of Leucocytes from Normal Subjects

Subject	Sex	Blood type	No. of experiments	Mean	S.D.
				mm.	*mm.*
KEK	F	A+	5	0.47	0.21
MMK	M	A+	37	0.61	0.19
JQH	F	A+	12	0.61	0.14
RK	F	O+	6	1.35	0.45
JL	M	A+	30	1.96	0.57
LS	F	O+	24	2.46	0.50
CBF	M	O+	9	2.67	0.52

from 1 to 3 times. The results are shown in Fig. 4. The subjects were laboratory or hospital personnel, medical students, etc., in good health.

These results indicate that the normal limits of leucocyte migration are wide. Those subjects whose leucocytes migrated less than 0.3 mm. or more than 3.5 mm. in the initial test, showed, on subsequent testing, migration rates falling between these limits. Apparently there are factors at work in the normal individual which may cause at least an occasional wide fluctuation in leucocyte migration.

While some individuals, such as those indicated in Table I, were shown to be consistent from day to day, other individuals could be found in whom wide fluctuations in migration distance were observed. Data from two such individuals are presented in Table II. In the light of our later experience with subjects undergoing symptomatic respiratory infections, as well as more serious illnesses, it is interesting that these two subjects had arrested tuberculosis, one of the spine, and the other of the lungs.

Neither an individual's day to day variation in leucocyte migration, nor the

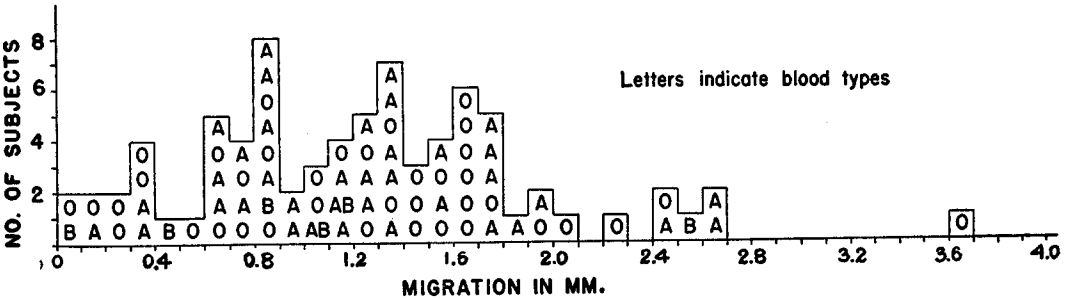

FIG. 4. Distribution of leucocyte migration distances in a series of 78 normal individuals.

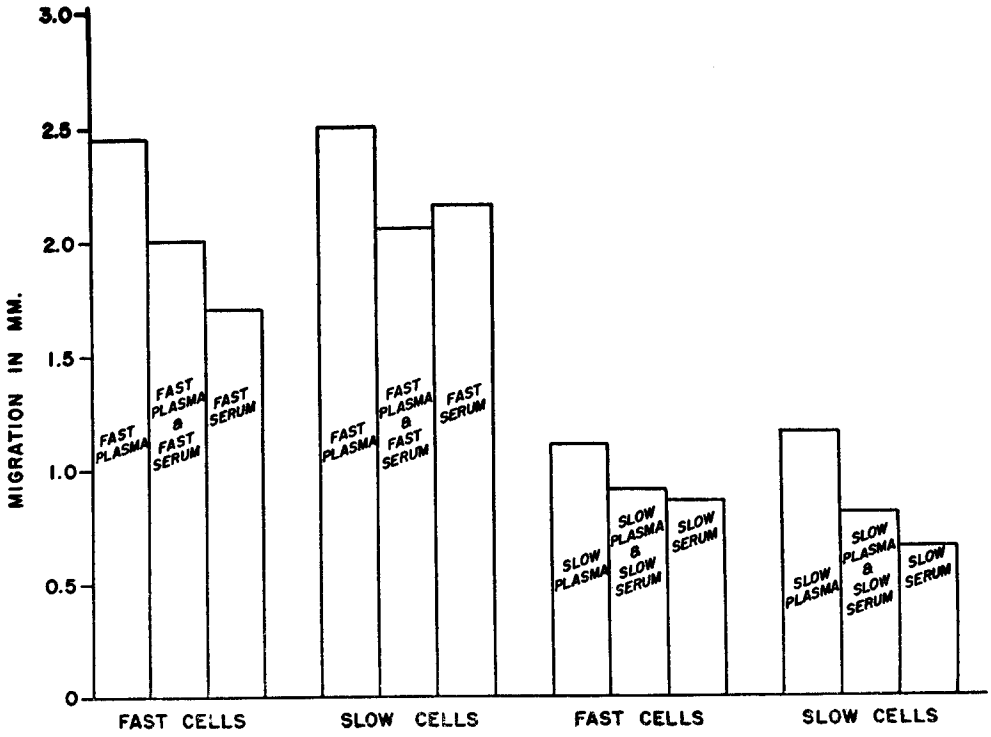

FIG. 5. Effect on leucocyte migration of the removal of fibrinogen from the plasma.

variation among individuals, could be correlated with the total or differential white blood count in whole blood or in the final cell suspension.

Leucocyte Migration during Illness.—An endemic virus-type respiratory illness manifested by coryza, malaise, fever, and mild bronchitis provided an opportunity to observe the effects of an infection on the leucocyte migration of several of the individuals listed in Table I who had already been studied to some degree. The early phase of these infections was frequently characterized by as much as a four- to fivefold increase in leucocyte migration. For a variable period thereafter, wide day to day fluctuations in leucocyte migration were

TABLE II

Average Migration Distances of Leucocytes from Tuberculous Subjects

Subject	Sex	Blood type	No. of experiments	Mean	S.D.
				mm.	*mm.*
LS	F	A+	32	1.61	0.76
FM	F	B+	49	1.46	0.65

TABLE III

Average Migration Distance of Leucocytes from Hospital Patients

Subject	Sex	Blood type	Disease	No. of experiments	Mean	S.D.
					mm.	*mm.*
RT	M	O+	Diabetes mellitus	13	1.33	0.62
EC	F	O+	Bronchitis	7	1.59	0.53
JK	M	A+	Myocardial infarction	6	1.68	0.76
WS	M	A+	Cerebrovascular accident	9	2.22	0.91
JB	M	O−	Bleeding duodenal ulcer with pyloric obstruction	14	2.39	0.80
SG	M	O−	Hyperparathyroidism	15	2.84	0.39

observed. These changes did not form a consistent pattern, and the return to normal was often slow.

Observations on more acute illnesses were made in a group of subjects hospitalized for a variety of disorders including infection and tissue insults (Table III). Although it was again found that leucocytes of some individuals migrated at a slow rate and others at a fast rate, there was distinctly more variation in each subject from day to day than was encountered in normal healthy individuals. This fact may be seen by a comparison of the S.D.'s of Table III (hospital patients) with the S.D. of Table I (normals). Since the S.D. is a function of the amount of variation in a group of data, a t test was applied to the S.D.'s of the normal group as compared with the S.D.'s of the

hospital patients to evaluate these differences in variation. The value for t is equal to 3.33, and p is less than 0.01. These results, coupled with the observations on normal subjects followed during the above mentioned virus infections, led to the conclusion that, during illness, the migration rate is more variable than in normal subjects.

The Effect of the Plasma on Leucocyte Migration.—We have noted in Table I the striking differences in the average leucocyte migration rate of various subjects. A series of experiments was undertaken to determine whether the variability was due to factors residing in the plasma or in the cells.

The protocol for these experiments is recorded in Table IV. For each experiment involving a "slow" and a "fast" migrator, 40 capillaries were prepared.

TABLE IV

Sample Protocol Combining Cell Suspensions in Autologous or Homologous Plasma

Subject I cells, *cc.*	0.2	0.2		
Subject II cells, *cc.*			0.2	0.2
Subject I plasma, *cc.*	0.3		0.3	
Subject II plasma, *cc.*		0.3		0.3
Hanks's solution, *cc.*	0.1	0.1	0.1	0.1
Results, *mm.*	3.0	0.4	3.2	0.6
	3.6	1.4	2.0	0.8
	3.1	0.9	2.7	0.4
	2.6	0.6	0.3	0.9
	3.9	0.4	1.9	0.1
	2.4	0.4	0.6	1.0
	1.8	1.2	1.3	1.1
	2.7	0.8	2.7	0.4
	2.7	1.1	2.5	0.7
	2.7	1.6	2.4	0.4
Mean *mm.*	2.8	0.9	1.9	0.6
S.D., *mm.*	0.6	0.4	0.8	0.3

10 contained slow cells in slow plasma; 10, slow cells in fast plasma; 10, fast cells in slow plasma; and 10, fast cells in fast plasma. The results of a series in which 7 pairs of subjects were studied in this manner in a total of 30 experiments are shown in Table V. Fast cells in slow plasma migrate almost as slowly (1.3 mm.) as do slow cells in slow plasma (1.0 mm.) and slow cells in fast plasma migrate almost as fast (1.7 mm.) as fast cells in fast plasma (2.2 mm.). It can therefore be concluded that while the cells have some influence on the migration rate, the major factors influencing cell migration reside in the plasma.

It should be pointed out, however, that while the majority of the experiments indicated that cell migration was under plasma control, in approximately 1 out of 15 experiments, the reverse seemed to be true. In these unusual instances, the major forces affecting cell migration appeared to be in the cells.

These findings could not be correlated with illness, with leucocyte counts either in the blood sample as it was taken from the patient or used in the experiment, nor with any other discernible cause.

The Nature of the Plasma Factor.—An extended series of investigations was undertaken to determine the nature of the plasma factor or factors involved in the control of leucocyte migration.

(*a*) *Isoagglutinins.* In experiments in which the plasma and cells were obtained from different individuals, major blood groups were ordinarily matched in order to eliminate isoagglutinins as a source of variability. In a series of experiments in which this matching was not performed, it was found that the plasma factor had similar effects on both autologous and homologous cells.

TABLE V

Average Migration Rate of Slow and Fast Cells in Autologous and Homologous Plasmas

Subjects		Blood types		No. of experiments	Slow cells and slow plasma	Fast cells and slow plasma	Slow cells and fast plasma	Fast cells and fast plasma
Slow	Fast	Slow	Fast					
					mm.	*mm.*	*mm.*	*mm.*
MMK	JL	A+	A+	8	0.6	1.0	1.6	2.1
MMK	JS	A+	A+	7	1.1	1.7	1.5	2.2
MMK	ES	A+	O+	5	0.9	1.2	1.5	2.3
FM	LS	B+	A+	4	1.1	1.1	1.9	2.1
BL	LS	O+	A+	2	1.2	1.5	1.6	1.8
JQH	LS	A+	A+	2	1.9	1.5	2.7	3.2
LA	JL	O+	A+	2	1.2	Clotted	1.8	2.2
Over-all averages*, *mm.*					1.0	1.3	1.7	2.2
S.D.* *mm.*					0.55	0.98	0.94	0.86

* Obtained by weighting each of the 30 experiments equally.

It is therefore evident that the plasma factor responsible for the rate of cell. migration is not an isoagglutinin.

(*b*) *Fasting vs. Non-Fasting Blood.* Samples of blood were taken before breakfast, and 1 hour after breakfast from the same individual. A comparison of the migration of prebreakfast cells in prebreakfast plasma with postbreakfast cells in postbreakfast plasma showed that fasting had no detectable effect on leucocyte migration under the conditions of the present technique.

(*c*) *Degree of Clotting.* We have seen that leucocytes from various sources cultured in a given plasma sample tend to migrate at a similar rate. To test the possibility that this was the result of a simple physical barrier in the plasma clot, samples of blood from a slow individual (MMK) and a fast individual (JS) were "whipped," and the serum recovered. Cells from the slow individual were cultured in slow and fast serum, and cells from the fast in-

dividual were cultured in slow and fast serum. Except for the substitution of serum for plasma, the protocol was the same as that in Table IV.

In the absence of a clot, the leucocytes migrated along the capillary tube walls. The results, as shown in Table VI, demonstrate that the factors controlling the rate of leucocyte migration are present in the serum as well as in the plasma.

(*d*) *The Suspension Stability of the Blood.* The experiments of Fahraeus (1929) indicate that the sedimentation rate of erythrocytes is affected by the relative amounts of certain plasma proteins, especially fibrinogen, present in the plasma. Although the sedimentation of erythrocytes and the active migration of leucocytes are very different phenomena, the possibility presented itself that both cell activities might be related.

Two blood samples were collected from a "slow" individual (MMK), and two blood samples from a "fast" individual (JS). One sample from each individual was heparinized as usual, and used as a source of cells and plasma.

TABLE VI

Average Migration Distance of Leucocytes Cultured in Slow and Fast Serum

Serum	MMK cells	JS cells
	mm.	*mm.*
MMK	0.95	1.15
JS	2.15	2.35

The other sample from each individual was "whipped" to remove the fibrinogen. As shown in Fig. 5, fast cells were tested in fast plasma, in fast serum, and in a mixture of equal parts of fast plasma and fast serum. Slow cells were tested in fast plasma, in fast serum, and in a mixture of fast plasma and fast serum. Fast cells were tested in slow plasma, in slow serum, and in a mixture of slow plasma and slow serum. And, slow cells were tested in slow plasma, in slow serum, and in a mixture of slow plasma and slow serum.

As shown in Fig. 5, the removal of fibrinogen resulted in some change in the absolute migration rate. Relative migration rates remained the same, indicating that the plasma factors, which caused cells to migrate faster in one plasma than another, were present in serum as well as in plasma.

(*e*) *Dialysis and Heating of the Plasma.* Samples of plasma from a "slow" migrator (MMK) and a "fast" migrator (JL), respectively, were dialyzed at 4°C. for 48 hours against three changes of 1000 volumes of Hanks's solution. Dialyzed plasmas and non-dialyzed control plasmas were then tested against slow and fast cells taken from the same individuals.

As shown in Table VII, dialysis of the plasma had the effect of decreasing the absolute migration rate. However, fast cells in dialyzed fast plasma mi-

grated faster (1.3 mm.) than fast cells in dialyzed slow plasma (0.7 mm.), and slow cells in dialyzed fast plasma migrated faster (1.2 mm.) than slow cells in dialyzed slow plasma (0.5 mm.). Plasma factors controlling leucocyte migration are not removed by dialysis.

An experiment was also performed in which the cells of slow and of fast individuals were tested against slow and fast plasmas which had been sub-

TABLE VII

Average Migration Distance of Cells Cultured in Untreated and in Dialyzed Plasmas

Plasma	JL cells	MMK cells
	mm.	*mm.*
JL untreated	2.1	1.7
MMK untreated	1.1	0.7
JL dialyzed	1.3	1.2
MMK dialyzed	0.7	0.5

TABLE VIII

Migration of Leucocytes in Untreated Plasma and in Plasma Heated at 56°C. for 30 Minutes

Migration distance		Decrease
Untreated	Heated	
mm.	*mm.*	*per cent*
1.3	0.5	62
2.6	0.6	77
2.4	0.8	67
2.0	0.1	95
2.2	0.4	82
1.6	0.3	81
Average		77

jected to a temperature of 56°C. for 30 minutes. As shown in Table VIII, this treatment drastically lowered the accelerating properties of the plasma, indicating that the plasma factors involved in leucocyte migration are "heat-labile."

(*f*) *Lack of Effect of Complement.*[6] The fact that heating the plasma to 56°C. for 30 minutes reduced the accelerating properties of the plasma suggested that complement might be involved. Attempts to increase the migration rate of cells in slow plasma by the addition of guinea pig plasma, which is noted

[6] The authors are indebted to Dr. Joseph J. Hoet, Peter Bent Brigham Hospital, Boston, for his assistance in carrying out these experiments.

for its high complement titer, were unsuccessful. Furthermore, decomplementing plasma by adsorption with successive exposures to sensitized sheep cells did not affect the plasma migration factor. When this complement-free serum was then heated at 56°C. for 20 minutes, the plasma factor was destroyed.

(*g*) *Fractionation of the Plasma.* In an effort to determine which plasma proteins exerted an effect on leucocyte migration, a series of experiments was performed with plasma protein fractions. These fractions were obtained by the use of the small volume, cold alcohol technique described by Lever *et al.* (1951), and modified in this laboratory by Vaughan *et al.* (1955). After their precipitation, the fractions were redissolved in Hanks's solution, brought back to ⅓ the volume of the original plasma, and dialyzed for 48 hours against several changes of Hanks's solution. Aliquots of the fractions were then stored

TABLE IX

The Effect on Leucocyte Migration of Adding Fractions of Plasma Proteins to Control Plasma

Control	Control + fraction II	Control + fraction III	Control + fraction IV + V
mm.	*mm.*	*mm.*	*mm.*
2.2	1.3	0.4	1.0
2.5	1.5	0.7	1.0
1.6	1.6	1.5	2.1
1.7	1.4	0.2	1.9
1.2	1.6	1.7	2.5
0.9	0.9	0.2	1.5
3.1	2.2	0.2	1.5
Average, *mm.* 1.9	1.5	0.7	1.6
S.D., *mm.* 0.8	0.3	0.6	0.7

at −20°C. until used. While there are many proteins present in plasma which are not yet described, or which are present only in traces, the separation of plasma proteins, according to this technique, is stated by Lever *et al.* to be as follows:—

Fraction II, γ-globulin.

Fraction III, β_1-lipoproteins, β_1-lipid-poor proteins, ceruloplasmin, isoagglutinins, plasminogen, cold-insoluble globulin, prothrombin, and part of the fibrinogen.

Fractions IV and V, serum albumins, β_1-metal-combining proteins, α_2 glycoproteins, α_2 mucoprotein, α_1 lipoprotein, iodoproteins, choline esterase, alkaline phosphatase, α_1 glycoprotein, α_2 protein, β_1-protein, other small proteins and peptides.

Table IX is a summary of 7 experiments in which the plasma protein fractions of an individual with slow leucocyte migration (MMK) were added to

the whole plasma of an individual with fast leucocyte migration (JL) of the same blood type (A, Rh+). The cells used were autologous to the whole plasma. The fractions were added to the control plasma in amounts which made the final concentration of the added fraction equal to ⅔ of the amount of that fraction contained in the control plasma. It can be seen from Table IX that the addition of fraction III caused an inhibition of the migration of leucocytes, while the other fractions exerted relatively little effect. The t test shows that the effect of fraction III was at the 0.01 level of significance, while the effects of fraction II and of fraction IV + V were not significant.

Two similar experiments were performed in which the plasma protein fractions were obtained from different sources. In one, we observed the migration of the leucocytes of an individual (JL) when the leucocytes were migrating in his own plasma to which had been added proteins fractionated from his own plasma. In the second experiment, we observed the migration of the leucocytes of an individual (JL, type A, Rh+ blood) when the leucocytes were migrating in his own plasma to which were added the proteins fractionated from the plasma of an individual (ES) of a different major blood group (Type O, Rh+). With the experiment described in the preceding paragraph, this made a series in which the cells and plasma were the same, but to which were added plasma protein fractions from three different sources; from the individual's own plasma, from the plasma of a different individual of the same major blood group, and from the plasma of an individual of a different major blood group.

In all of these experiments, the results were in agreement with those recorded in Table IX.

It will be noted that fraction III acted as an inhibitor of leucocyte migration. But the above-mentioned experiments in which plasma was heated to 56° for 30 minutes indicated that a heat-labile stimulator might be involved. No consistent evidence was encountered in any of the plasma fractions for a factor which accelerated migration in whole plasma although in a few experiments only fraction II had a stimulatory effect. This is a different finding from earlier experiments in this series in which a heat-labile plasma factor was studied. Further information on this point was obtained when plasma protein fractions were added to plasma which had previously been heated to 60°C. for 30 minutes. The fractions were obtained either from type A, Rh+ or type O, Rh+ individuals. In each experiment, one series was prepared with type A fractions, and one series with type O fractions.

The results of these experiments are recorded in Table X. It can be readily observed that the addition of any of these plasma protein fractions caused a significant change in leucocyte migration. The addition of fraction III and of fraction IV + V caused a decrease in migration rate. The addition of fraction II caused an increase in leucocyte migration. In an effort to verify the fact

that the addition of fraction II was replacing a stimulating factor that had been destroyed by the heating of whole plasma, samples of fraction II, heated to 60°C. for 30 minutes, were included in the later experiments. As indicated in Table X, heated fraction II was not able to significantly alter leucocyte migration when added to heated plasma. This final result indicates that the

TABLE X

Effect on Leucocyte Migration of Adding Fractions of Plasma Proteins to Control Serum which had been heated to 60°C. for 30 Minutes

Heated control	Control serum	Δ*	Heated control + fraction II	Δ*	Heated control + heated fraction II	Δ*	Heated control + fraction III	Δ*	Heated control + fraction IV + V	Δ*
mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
1.1	1.6	0.5	1.6	0.5			1.3	−0.2	1.0	−0.1
			1.5	0.4			0.8	0.3	0.8	−0.3
0.8	1.8	1.0	1.2	0.4			0.8	0.0	0.8	0.0
			1.2	0.4			0.9	−0.1	0.8	0.0
0.7	1.1	0.4	0.8	0.1			0.3	−0.4	0.5	−0.2
			0.8	0.1			0.3	−0.4	0.5	−0.2
0.8	1.0	0.2	1.0	0.2			0.7	−0.1	0.6	−0.2
			1.0	0.2			0.7	−0.2	0.5	−0.3
0.5	0.7	0.2	0.4	−0.1	0.4	−0.1	0.6	−0.2	0.3	−0.2
			0.7	0.2	0.6	0.1	0.3	−0.2	0.4	−0.1
1.5	2.7	1.2	1.9	0.4	1.4	−0.1	1.3	−0.2	1.2	−0.3
			2.3	0.8	1.7	0.2	1.4	−0.1	1.0	−0.5
Mean		0.6		0.3		0.02		−0.2		−0.2
S.D.		0.42		0.2		0.17		0.2		0.14
S.E.M.		0.17		0.06		0.09		0.06		0.04
Mean/S.E.M (C.R.)		3.5		5.0		—		5.3		5.0

* Difference between heated control, and heated control with fraction added.

accelerator in fraction II is characterized by the same heat lability as that in whole plasma.

These experiments demonstrate the presence in the plasma of at least two factors which regulate leucocyte migration; a heat-labile accelerator found in fraction II and an inhibitor found in fraction III.

DISCUSSION

In the experiments described above, a single aspect of the physiology of peripheral blood leucocytes has been under observation; namely, that of

leucocyte migration. Whether enhanced migration is correlated with other functions of leucocytes is not the subject of this report. Present evidence indicates that the host possesses a homeostatic humoral mechanism, consisting of an inhibitor and a stimulator, which during some types of stress may cause wide fluctuations in the migratory rate of leucocytes.

The major factors which influence leucocyte migration are contained in the plasma (Table V). Under the conditions of these experiments the cells themselves account for some of the difference in migration distance among individuals. Stated briefly, fast cells in slow plasma migrate faster than slow cells in slow plasma, and slow cells in fast plasma migrate slower than fast cells in fast plasma. It may be that washing leucocytes completely free of adsorbed or ingested plasma is impossible, especially after they have been subjected to repeated mild trauma. Perhaps "washed" cells are, to a certain extent, still under the influence of traces of the plasma from which they were taken. Cell factors may be due to other causes as well.

C-reactive protein, which appears in the plasma of individuals in disease states, and is not ordinarily present in the plasma of normal individuals, was reported by Wood (1951) to increase leucocyte migration. We have confirmed the essentials of his results. Using our capillary tube technique, high concentrations of C-reactive protein[7] inhibit leucocyte migration. Optimal concentrations stimulate leucocyte migration 30 to 40 per cent. Since normal subjects do not possess detectable amounts of C-reactive protein in their blood, the plasma factor of this study does not appear to be the same phenomenon described by Wood.

Menkin (1950) has postulated the existence of a leucocyte-promoting factor (LPF), thought to be a globulin or polypeptide, derived from tissue exudates. According to Menkin, this factor is present only where tissue exudates occur. It is doubtful that LPF bears any relation to the plasma factors here described.

In a study of the migration of leucocytes from an explant, Allgower and Sullman (1950) found that diminished migration takes place in Tyrode's solution alone whereas plasma proteins alter cell migration. By the use of a salting-out technique, they divided the plasma proteins into two fractions, the γ-globulins and the "rest-proteins." When the γ-globulins were added to the Tyrode's solution, it was found that the leucocytes migrated away from the explant to a much greater degree than when they were in Tyrode's alone, or in Tyrode's plus the "rest-proteins." It was also found that the γ-globulins exerted a positive chemotactic effect on leucocytes. Taken together with our finding that fraction II, which contains the γ-globulins, enhanced the migration of leucocytes, these results strongly suggest that the γ-globulins have a function in disease resistance beyond that associated with antigen-antibody reactions.

[7] C-reactive protein for these experiments was kindly supplied by Dr. Harrison F. Wood of the Hospital of The Rockefeller Institute, New York.

The utilization of this *in vitro* technique has provided an opportunity to investigate the effects of plasma in the regulation of leucocyte migration. While the conditions inside a capillary tube are unlike those in the living host, it is quite likely that this laboratory procedure accurately reflects *in vivo* mechanisms controlling the rate of leucocyte migration. Further work will be necessary to determine the extent to which this plasma system controls other functions of leucocytes, and whether it is important in the pathogenesis of disease processes involving leucocytes.

SUMMARY

A new method is described for the quantitative study of leucocyte migration. Blood drawn into a capillary tube is centrifuged, forming a buffy coat. The migration of the leucocytes from the buffy coat into the plasma is measured by means of an ocular micrometer.

While there is a great deal of variation from one normal individual to another in the migration rates of leucocytes those of each individual show relatively little variation in migration from day to day (Table I).

In all cases of illness studied, the average migration rates fall within the normal range, but there was a wider variation in the day to day migration rate than obtains in normal healthy individuals (Tables II and III).

From experiments in which the cells with typically "slow" and "fast" migration rates were tested in both the "slow" and "fast" plasmas, it could be concluded that while the cells invariably have some influence on the migration rate, the major factors influencing leucocyte migration reside in the plasma (Table V).

Experiments in which the plasma proteins were fractionated according to the method of Lever *et al.* indicated the presence of a system of proteins which regulate leucocyte migration. A heat-labile (56°C. for 30 minutes) component of fraction II was able to accelerate leucocyte migration, while fraction III acted as an inhibitor (Tables IX and X).

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