

Marrow Cell Egress

THE CENTRAL INTERACTION OF BARRIER PORE SIZE AND CELL MATURATION

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ABSTRACT Release of marrow cells may be determined primarily by the restrictive barrier that separates marrow hematopoietic cords from sinusoids, and by the ability of the cell to negotiate the barrier pores which are of smaller diameter than the cell. This critical interrelationship may be further modulated by humoral agents (releasing factors).

To test this hypothesis, we placed human marrow cells in a chamber between millipore or nucleopore filters with pore diameters of 1–8 μm . Fixed, stained, cross sections of the filters allowed histological examination of the penetration of cells and quantification of egress of age-specific cell types. The rate of marrow granulocyte egress was highly correlated with (a) barrier pore diameter, (b) morphological age of cells, and (c) the presence of a chemical attractant. Immature granulocytes would not exit through a restrictive barrier even after protracted periods and were not responsive to chemoattractants. Intermediate-aged granulocytes showed a slight ability to respond to attractants and to exit if pore diameters were large. Mature granulocytes exited through the restrictive barrier at all pore diameters studied, however, this egress was accelerated by increasing pore diameter and by the presence of an attractant. Leukemia blast cells were incapable of traversing pore diameters of 1–8 μm .

These studies support the hypothesis that the development of deformability, motility, and surface receptors for chemoattractants at the later stages of granulocyte development allow the egress of cells through

the marrow sinusoid wall which appears by electron microscopy to be a porous barrier with aperture diameters smaller than cell diameters; and that this process can be modulated by humoral agents which enhance directed movement of cells and may also increase pore size. Moreover, on the basis of our observations, the egress of leukemia cells is best explained by destruction of the normal sinusoid barrier of marrow indicating that manifestations of the disease are dependent on alteration in stromal as well as parenchymal marrow cells.

INTRODUCTION

The regulation of release of marrow cells in normal and pathological states is poorly understood. Studies of the ultrastructure of marrow have indicated that a barrier separates hematopoietic cells from marrow sinusoids and that pores or potential pores in the barrier have a smaller diameter than do hematopoietic cells (1–5). Recent studies of deformability of marrow cells have indicated that immature granulocytes (6, 7) and erythrocytes (8) lack whereas, mature granulocytes and erythrocytes achieve the ability to traverse microcapillary tubes with diameters smaller than that of the cell. This has suggested that (a) the porosity of the marrow sinusoid wall and (b) the biophysical properties of maturing hematopoietic cells may be two central factors governing marrow release. Superimposed on this central relationship would be the effect of humoral factors to accelerate cell egress depending on need (9–11). When the steady state is disturbed, the provision for the immediate need of additional cells would be the result of accelerated delivery of mature cells from marrow, followed later by the continuous provision of increased

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numbers of mature cells as a result of an increased production and maturation rate (12, 13).

In order to test the hypothesis that the degree of cell maturation and marrow pore size could interact to influence rate of cell egress, we devised an *in vitro* marrow analogue which allowed the examination of cell migration through filters containing pores with dimensions similar to those observed in electron micrographs of marrow. Moreover, the preparation of "histologic" cross sections of filters allowed the identification of individual marrow cells at any depth within the filter; and, thereby, the quantification of migration of marrow granulocytes of specific morphologic age could be made.

The effect of a granulocyte attractant, complement activated by the action of endotoxin on fresh plasma, on the migration of marrow granulocytes of different morphologic age through barriers of different pore size was also examined. This permitted the assessment of the relationship of three variables, (a) cell maturity, (b) barrier pore size, and (c) humoral attractant in determining egress.

METHODS

Preparation of cell suspensions. Blood from healthy subjects and marrow from subjects without disorders of leukopoiesis and blood and marrow from untreated subjects with acute granulocytic leukemia were collected in sodium heparin (14.3 $\mu\text{m}/\text{ml}$) and allowed to sediment at 1 *g* at room temperature without addition of a red cell-sedimenting agent. The upper portions of the tube in which sedimentation was performed was carefully wiped clean of blood with a moist applicator stick. The leukocyte-rich plasma was removed and contained a leukocyte to erythrocyte ratio of about 4 to 1. In subjects with acute leukemia, the cell suspension was placed onto a column composed of a 3 ml plastic syringe containing 5 g of glass beads, 0.45–0.50 mm in diameter (Glasperlens, VWR Scientific Div., VWR United Corp., San Francisco, Calif., Rochester, N. Y.) for 15 min at 37°C. Nonadherent cells were eluted with 3 ml of autologous or blood group compatible isologous plasma delivered through a 26 gauge needle from a 5 ml plastic syringe (7). Normal marrow cell suspensions were divided into two samples, one of which was filtered through a column of glass beads at 37°C as described above. Filtration consistently resulted in a preparation that had more than 95% leukemic blast cells in the samples from the subjects with acute leukemia and less than 5% polymorphonuclear neutrophils and band neutrophils from samples of normal marrow. Companion filtered and nonfiltered marrow-cell suspensions were studied simultaneously. All samples were diluted with autologous plasma to a cell concentration of $6\text{--}9 \times 10^8$ cells/ mm^3 . In the rare circumstance where cell suspensions had a lower cell density, no adjustment was made.

Cell counts were performed on a celloscope particle counter (Particle Data, Inc., Elmhurst, Ill.) using a 76 μm orifice.

In order to examine the effect of red cells on white cell migration through filters, experiments were performed at red cell to white cell ratios of 1:1 and 1:50. For the latter condition, leukocyte-rich plasma prepared from marrow samples was centrifuged at 1200 rpm for 12 min. The plasma

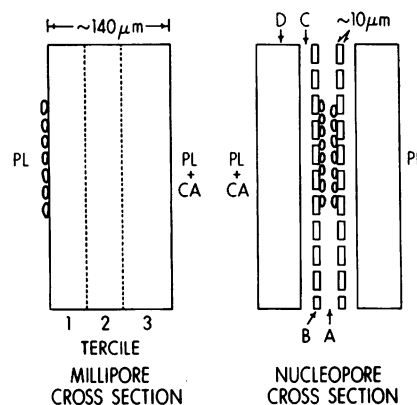


FIGURE 1 A schematic diagram of the cross section of (a) millipore filter (left) indicating the terciles arbitrarily selected to enumerate migration, and of (b) the quadripartite arrangement of nucleopore filters (B) overlaid with millipore filters (D) to trap cells which egress through nucleopore filters. The four-ply arrangement was held together with silver surgical clips. The interfilter cavity (A) containing marrow cells is analogous to hematopoietic cords. The spaces (C) between the nucleopore and millipore filters are analogous to vascular sinusoids.

was aspirated and the cells were resuspended in 10 mM tris-(hydroxymethyl)aminomethane hydrochloride-buffered 150 mM ammonium chloride, pH 7.48, 300 mosM, for 5 min, and the cell suspension was centrifuged at 1200 rpm for 7 min (14). The supernate containing red cell ghosts was discarded, and the sediment of leukocytes was washed once with and resuspended in plasma. This technique provided cell suspensions with less than 2% erythrocytes. Measurement of leukocyte migration through millipore filters was examined as noted below.

Millipore filter migration studies. 20 ml of cell suspension was centrifuged in a Shandon cytocentrifuge (Shandon Southern Instruments Inc., Sewickley, Pa.) at 500 rpm for 5 min onto millipore filters (Millipore Corp., Bedford, Mass.) of specific mean pore diameter as described by Baum, Mowat, and Kirk (15). The pore diameters used were 1.2, 3.0, 5.0, and 8.0 μm . Millipore filters were placed in Sykes-Moore chambers (Bellco Glass, Inc., Vineland, N. J.) in which the filter was supported with specifically fitted rubber gaskets (Chamberlin Rubber Co., Rochester, N. Y.) thereby separating the chamber into two compartments.

Heparanized autologous or blood group-compatible isologous plasma was introduced into the upper side of the chamber on the side of the filter to which cells had been applied. The lower chamber was filled with either plasma or plasma to which casein (Difco Laboratories, Detroit, Mich.) at a final concentration of 3.3 mg/ml was added. Each experiment was performed with matched chambers containing plasma alone or plasma with casein at each pore size to be studied.

After incubation at 37°C at times necessitated by individual experiments, the filters were removed from the chambers, fixed for 30 min with Helly's solution (95% Zenkers and 5% concentrated formalin), washed three times in distilled water, and placed in 70% ethyl alcohol. Filters were then imbedded into paraffin blocks, and 4- μm sections were made through the center of the cell pellet using an American Optical rotary microtome, (American Optical Corp.,

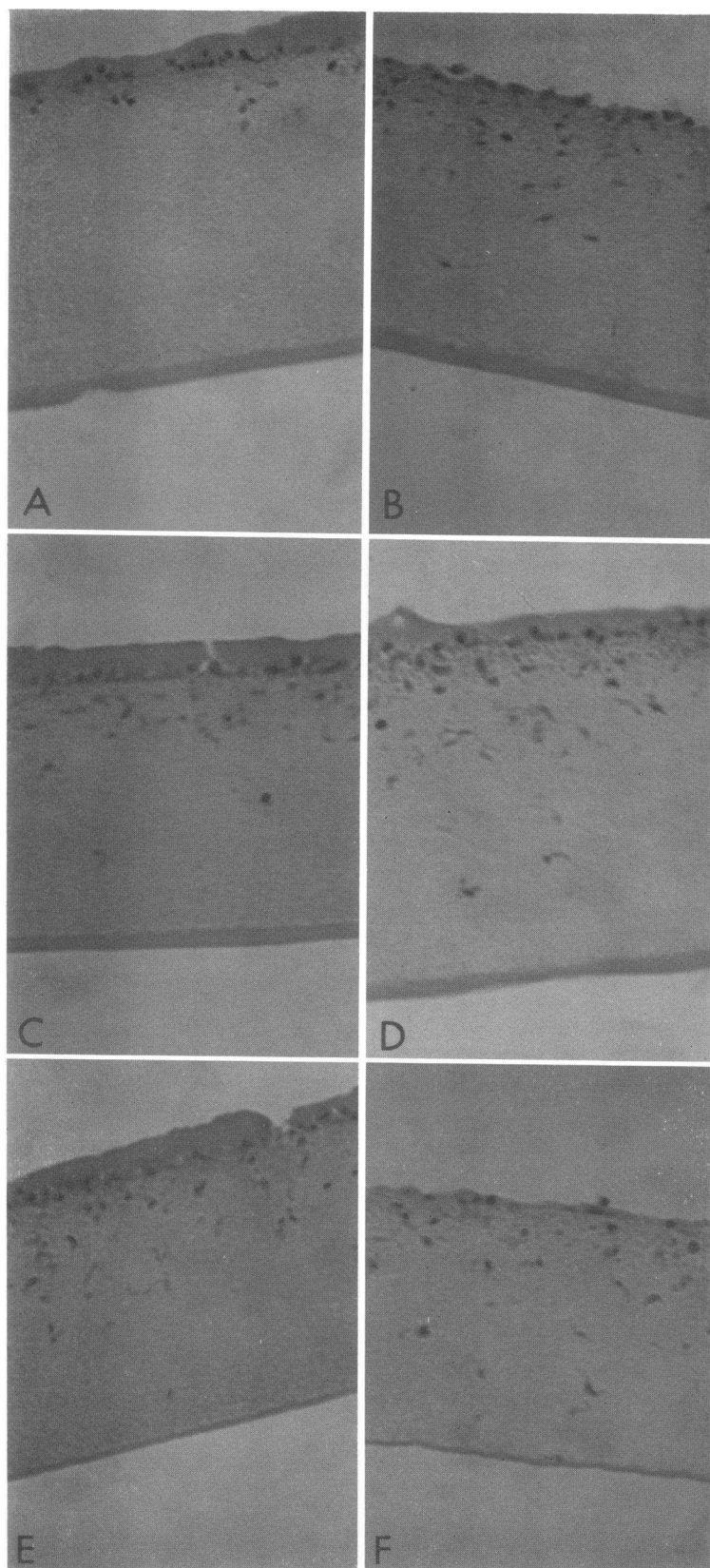


TABLE I
Migration of Normal Marrow Granulocytes

Depth of migration (zone)	Millipore filter mean pore diameter							
	1.2 μ m		3.0 μ m		5.0 μ m		8.0 μ m	
	PI	PI + Ca	PI	PI + Ca	PI	PI + Ca	PI	PI + Ca
I. 0-39 μ m	94 \pm 1.5 (89-98)	81 \pm 6.5 (63-92)	87 \pm 2.1 (82-92)	70 \pm 4.0 (63-79)	87 \pm 1.7 (82-90)	64 \pm 7.3 (49-84)	85 \pm 0.9 (82-86)	74 \pm 4.7 (65-83)
II. 40-78 μ m	4 \pm 1.6 (2-6)	10 \pm 2.1 (5-15)	9 \pm 3.3 (5-12)	18 \pm 3.4 (7-22)	8 \pm 2.3 (3-14)	12 \pm 2.3 (6-17)	10 \pm 2.1 (2-15)	15 \pm 1.3 (13-19)
III. >78 μ m	2 \pm 0.9 (0-5)	9 \pm 5.7 (3-26)	4 \pm 1.6 (1-8)	12 \pm 4.7 (7-28)	5 \pm 2.4 (3-8)	24 \pm 7.8 (4-39)	5 \pm 1.4 (2-8)	11 \pm 3.6 (4-18)

Mean \pm SE and the range in parenthesis of four experiments. Values represent the percent of cells in each tercile of depth at 3 h of incubation at 37°C. Since a total of 300 cells were counted on each filter, the absolute number of cells in each tercile can be calculated by $\% / 100 \times 300$, or $3 \times \%$. PI, plasma; Ca, casein.

Scientific Instrument Div., Buffalo, N. Y.). The resultant histologic sections were stained with hematoxylin and eosin.

For the purposes of quantifying cell migration, the millipore filters were divided into three zones by depth extending from the surface to which the cells had been applied. Zone I was 0-39 μ m, zone II was 40-78 μ m, and zone III was any distance greater than 79 μ m in depth from the surface (Fig. 1). The third zone varied from 51-71 μ m in width due to variation in total thickness of filters from 130 to 150 μ m. The slides containing stained cross sections of the filters were coded and counted by an observer who was unaware of the conditions of this study. 300 cells were counted on each cross section of the filter. A Leitz microscope (E. Leitz, Inc., Rockleigh, N. J.) with a calibrated eyepiece micrometer was used to determine depth zones. Individual cell detail was determined with an oil immersion lens (magnified $\times 1200$). Results have been expressed as a percent of cells in zones I, II, and III.

The influence of centrifugal force on cell penetration into the filters was examined by fixing preparations immediately after the application of cells to the filter with the cytocentrifuge and quantifying penetration into the filter on histological cross sections as described above.

Nucleopore filter migration studies. Further experiments were performed using nucleopore filters (General Electric Co., Pleasanton, Calif.) with pore diameters of 2, 3, 5, and 8 μ m and an average filter thickness of 10 μ m. Cell suspensions were applied to each of two nucleopore filters with the same pore diameter using the cytocentrifuge. The two filters were then placed with the surface containing the cell

button in apposition. To the outside of each nucleopore filter a millipore filter was apposed (Fig. 1). This quadripartite structure was held together with silver surgical clips to prevent slipping. This structure was inserted into the Sykes-Moore chamber in the same fashion as were the millipore filters. One side of the chamber was filled with heparanized autologous plasma and the other side with the plasma containing casein. The effect of the presence and absence of a chemoattractant could be examined in the same chamber under identical incubation conditions. Chambers were incubated at 37°C for 15 or 60 min at which time the filters were removed from the chamber, fixed in 1% glutaraldehyde in phosphate-buffered NaCl, rinsed three times in phosphate-buffered NaCl, and resuspended in that buffer. The filters were then imbedded and cross sections prepared in a similar fashion to the millipore filters. The filters were examined to evaluate the effect of pore size and chemoattractant on migration.

RESULTS

In studies of unfiltered normal marrow-cell suspensions, 6% of marrow granulocytes penetrated into zones II and III of millipore filters with pore diameters of 1.2 μ m in the absence of an attractant after 3 h of incubation (Table I). Migration of marrow granulocytes in the absence of an attractant increased with increasing pore size although only minor enhancement of random migration was observed between a pore diameter of

FIGURE 2 Cross sections of millipore filters are shown. Panels A, C, and E depict marrow cells applied to filters with mean pore diameters of 1.2, 3.0, and 5.0 μ m, respectively and in which lower portions of the chamber was charged with plasma alone. Panels B, D, and F represent filters of 1.2, 3.0, and 5.0- μ m mean pore diameters, respectively, in which the lower portion of the chamber was filled with plasma containing casein (chemoattractant). Increased migration was evident in this study as pore size increased in the absence of a chemoattractant as demonstrated in the left hand panels, top to bottom. The effect of attractants can be seen by comparing the right hand panels containing attractant with the filter of similar pore diameter in the left-hand column which does not contain attractant. The thin band on the upper and lower surface of the filter represents a layer of plasma which often adheres to the filter. Variation in filter thickness is evident and was present within the same lot of filters. The variations in filter thickness had a range of 128-148 μ m in this experiment. (Original magnification $\times 250$.)

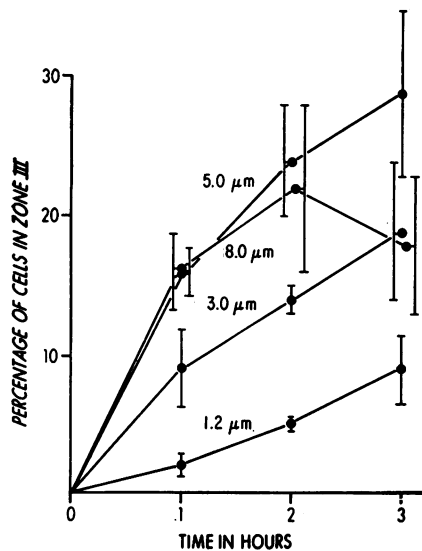


FIGURE 3 The percentage of peripheral blood granulocytes in zone III of millipore filters of mean pore size, 1.2, 3.0, 5.0, and 8.0 μm at 1, 2, and 3 h is shown. An increase in the rate of entry of cells into zone III occurred as pore size increased. However, the proportion at 3 h was the same or decreased in some 5 and all 8- μm filters. Using $\text{Na}^{51}\text{CrO}_4$ -labeled leukocytes, this change was shown to be due to the penetration and detachment of rapidly migrating granulocytes from the bottom of the filter.

3–8 μm . In the presence of chemoattractant (casein as an endotoxin source and plasma as a complement source) a marked increase in the migration of cells into zones II and III occurred at each pore size. At 1.2 μm , a threefold increase; at 3.0 μm , a 2.3-fold; at 5.0 μm , a 2.7-fold increase; and at 8.0 μm , a 1.7-fold increase in the proportion of cells in zones II and III was observed in the presence of an attractant (Table I). Moreover, an increase in the proportion of cells in zones II and III

occurred in the presence of an attractant as pore diameter increased. Hence at 3.0 μm , 30% and at 5 μm , 36% of cells were in zones II and III as compared with 19% at 1.2 (Table I). An example of such an experiment is depicted in Fig. 2. The proportion of cells in zones II and III increased with increasing pore diameter and was also accelerated by the presence of an attractant. Variation in filter thickness, which ranged from about 130 to 150 μm , did not effect quantification since the proportion of cells in the third tercile was based on cells which migrated over 78 μm from the surface. The reduction in the proportional increase in migration in the presence of an attractant at 3 h on 8- μm filters was shown to be due to the penetration of cells through the lower surface of the large pore size filters and detachment. This was established in two ways. Peripheral blood leukocytes were applied to filters with pores of 1.2–8.0 μm in diameter and studied at 1, 2, and 3 h. As shown in Fig. 3, the proportion of leukocytes in the third zone increased in a near linear fashion over 3 h, when filters of small pore size and more restricted migration rates were used. At large pore diameters, the percentage of leukocytes in zone III in individual experiments increased for 2 h and either reached an apparent steady state or decreased at 3 h. The extent of the decrease related, in part, to filter thickness. More third tercile cell loss occurred at 3 h if cells were applied to 120 as compared to 150- μm filters of 8 μm diameter pore size. If either retrograde migration of cells or preferential cell lysis in the third zone was present at 3 h to explain the smaller increment in zone III cells, such differences would have been expected to have affected studies regardless of filter pore size. Furthermore, studies were performed with $\text{Na}^{51}\text{CrO}_4$ -labeled leukocytes applied to the apposing surface of two millipore filters of small (3 μm) and large (8 μm) pore diameter.

TABLE II
Migration of Filtered Normal Marrow Granulocytes

Depth of migration (zone)	Millipore filter mean pore diameter					
	3.0 μm		5.0 μm		8.0 μm	
	Pl	Pl + Ca	Pl	Pl + Ca	Pl	Pl + Ca
I. 0–39 μm	99 \pm 1.2 (99–100)	97 \pm 2.7 (94–100)	97 \pm 2.6 (95–100)	92 \pm 7.5 (83–100)	96 \pm 3.5 (92–100)	88 \pm 9.0 (76–98)
II. 40–78 μm	1 \pm 1.0 (0–3)	2 \pm 0.9 (0–4)	1 \pm 0.5 (0–2)	5 \pm 1.8 (0–9)	2 \pm 2.2 (0–5)	6 \pm 2.4 (1–12)
III. >78 μm	0 (0)	1 \pm 0.6 (1–3)	2 \pm 0.7 (0–3)	3 \pm 2.0 (0–8)	2 \pm 0.6 (0–3)	6 \pm 2.5 (1–12)

Mean \pm SE and the range in parenthesis of four experiments. Values represent the percent of cells in each tercile at 3 h of incubation at 37°C. Since a total of 300 cells were counted on each filter, the absolute number of cells in each tercile can be calculated by $\% / 100 \times 300$, or $3 \times \%$. Pl, plasma; Ca, casein.

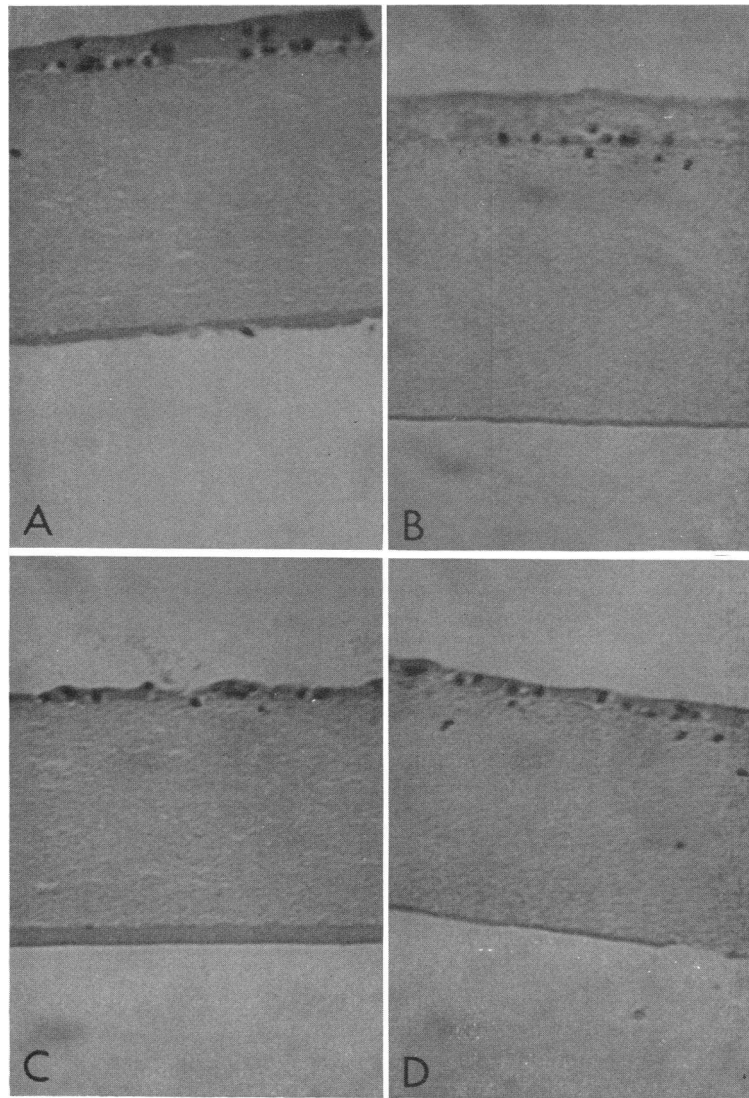


FIGURE 4 Filtered marrow from the same specimen as depicted in Fig. 2. Panels A and B depict cross sections of filters with 3.0- μm pore diameters with plasma (A) and plasma plus casein (B) in the lower section of the Sykes-Moore chamber. Panels C and D represent filters with an 8.0 μm mean pore diameter with plasma (C) or plasma and casein (D) in the lower portion of the chamber. The upper and lower surfaces of the filter are coated with a layer of plasma. On the upper side of the filter, cells are resting between the plasma layer and filter surface. No migration of immature cells was evident although an occasional cell advanced into the filter at large pore diameters and in the presence of casein (D). The latter cells could be identified as intermediate-aged granulocytes (late (small) myelocytes, meta-myelocytes, or band neutrophils). (Original magnification $\times 250$.)

Filters were placed outside of the filters to which cells were applied so that cells migrating through the filters of application were caught on the underlying or outer millipore filters. Cell transit was identified by measuring radioactivity of the outer "cell-catching" filters (data not shown). Radioactivity above background was not present at 1 h and occurred minimally at 2 h, however,

was definitely present at 3 h from cells placed on the large but not the small pore diameter filter.

Direct identification of cells in the cross sections of the filters could be made in most cases by microscopic examination of individual cells. Cells migrating to zones II and III were identifiable as band or segmented neutrophils. Rarely, cells which appeared to be metamyelo-

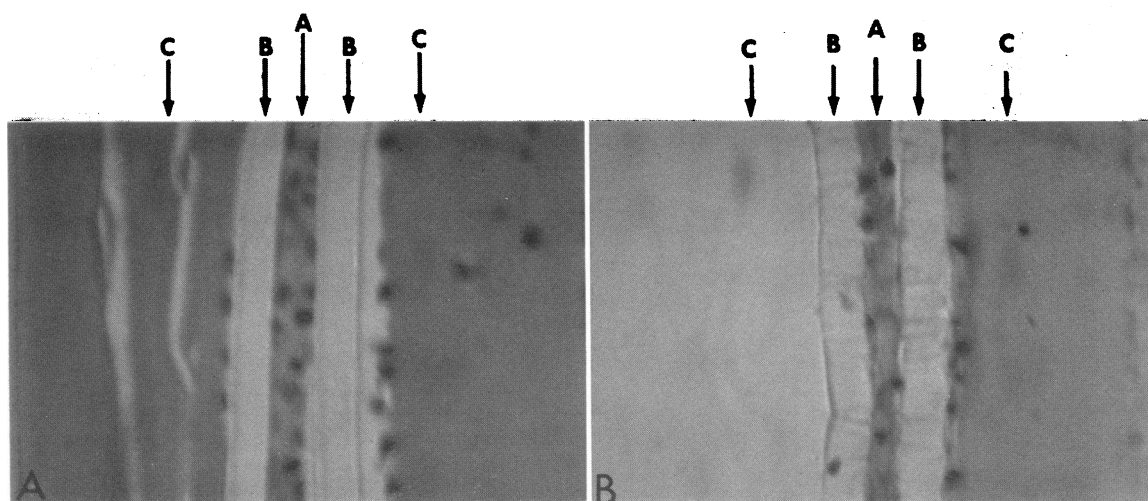


FIGURE 5 Two experiments are shown in which two nucleopore filters (B) were placed in apposition with normal marrow cells in the interfilter cavity (A). The millipore filter outside the field on the right was underlain with plasma and casein and the millipore on the left with plasma alone. Rapid egress of mature cells into the space (C) between nucleopore and millipore filter primarily occurred toward the side with attractant (right side). Immature cells were retained in the interfilter cavity (A). The filters in experiment A had pore diameters of $5.0\ \mu\text{m}$ and in experiment B had pore diameters of $8.0\ \mu\text{m}$. (Original magnification $\times 250$.)

cytes were seen. In confirmation of this observation, marrow which had adherent (mature) cells removed by filtration through glass beads (7), showed a marked reduction in the proportion of cells migrating to zones II and III (Table II). Indeed, the cells which did migrate to zone III were identified as residual band or segmented neutrophils. Cells which appeared to be of intermediate maturity (myelocytes, metamyelocytes) occasionally entered the deeper regions of zone I or into zone II. Blasts and progranulocytes did not leave the filter surface (Fig. 4). It is of interest that when filtered marrow containing cells of slower migration rate was studied, the proportional increment in migration into zones II and III in the presence of chemoattractant increased progressively (3, 8, and 12%), as

TABLE III
Migration of Leukemic Blast Cells

Depth of migration (zone)	Millipore filter mean pore diameter			
	$1.2\ \mu\text{m}$	$3.0\ \mu\text{m}$	$5.0\ \mu\text{m}$	$8.0\ \mu\text{m}$
I. $0-39\ \mu\text{m}$	100	99 (97-100)	100	99 (98-100)
II. $40-78\ \mu\text{m}$	0	1 (0-3)	0	1 (0-2)
III. $>78\ \mu\text{m}$	0	0	0	0

Mean and range of the percent of cells in each tercile in experiments performed on cells from five subjects with acute granulocytic leukemia.

filter pore size increased (3, 5, $8\ \mu\text{m}$) (Table II). In this situation, cell loss on large pore diameter filters was not present, and chemoattractant increased migration approximately threefold at each pore diameter. Chemoattractant had no effect on the ability of myeloblasts, progranulocytes, and early, large myelocytes to enter the filter interstices.

The removal of virtually all red cells, from cell suspensions, by prior osmotic lysis with Tris-buffered ammonium chloride did not enhance migration of leukocytes through filters when compared with cell suspensions containing larger proportions of red cells. This was expected since the high ratio of pores to cells made the impedence of leukocyte migration by red cells under these conditions unlikely.

Since egress of cells from marrow or from the circulation probably requires negotiation of pores which are of shorter length than the thickness of the millipore filter, studies were conducted with nucleopore filters whose thickness is $\frac{1}{4}$ th that of the millipore filter (Fig. 1). Moreover, the pore diameters have very uniform size distribution. When marrow cells were placed between nucleopore filters, cell migration was rapid (being accomplished in less than 15 min). However, migration was clearly related to maturity since mature granulocytes rapidly exited, whereas immature granulocytes could not egress even through nucleopore filters of $8\ \mu\text{m}$ diameter pore size. As was the case in studies with millipore filters, the presence of chemoattractant on one side of the quadripartite chamber caused more rapid

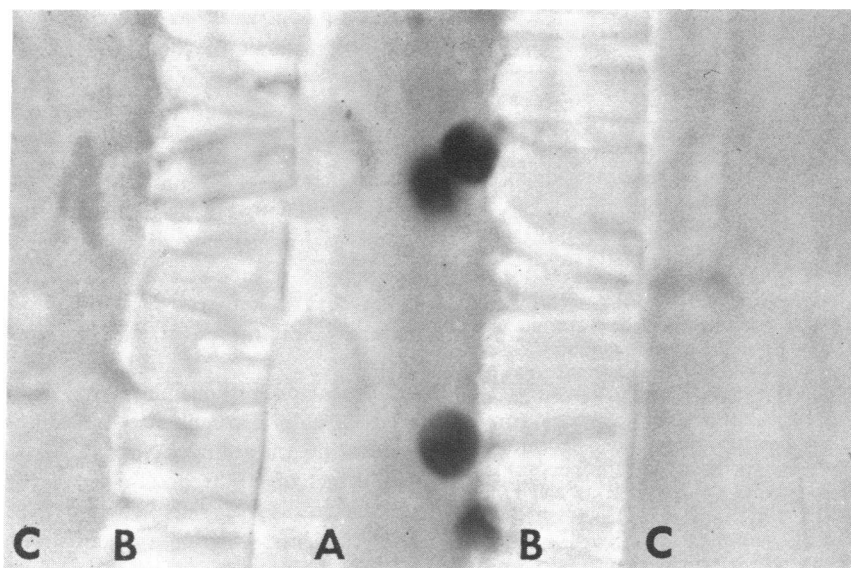


FIGURE 6 Marrow leukemic blast cells were placed in the cavity (A) created by the apposition of two nucleopore filters (B). The nucleopore filters were covered with millipore filters forming a quadripartite structure (see Methods). The millipore filter on the right was underlain with plasma and casein and on the left with plasma alone. Cells can be seen abutting pores in the nucleopore filters (B), but do not egress, even after 24 h observation. Nucleopore filter pore diameter is $3.0\ \mu\text{m}$ and cell diameter $8.1\ \mu\text{m}$ in the example shown. Similar results were observed when nucleopore filters with 5.0 and $8.0\text{-}\mu\text{m}$ pore diameters were used. Leukemic myeloblasts from blood or marrow did not traverse filters and enter the space (C) between nucleopore and millipore filters. Millipore ("cell-catching") filters are outside the field of this photomicrograph. (Original magnification $\times 1000$.)

and directed egress as compared with the opposite side of the chamber without attractant (Fig. 5). Band and segmented neutrophils could be observed in various phases of egress within pore channels. Immature granulocytes remained confined to the central cavity.

Marrow and blood leukemic myeloblasts were studied as to their ability to traverse restrictive barriers. Leukemic blast cells were unable to migrate into millipore filters, even at large pore sizes (Table III). Blast cells were virtually confined to the edge of application and a few microns below. Leukemic blast cells did not demonstrate any responsiveness to chemoattractant. Studies also indicated that it was difficult to maintain leukemic myeloblasts on the filter since they are nonadherent and make no firm contacts. However, at $8\text{-}\mu\text{m}$ pore diameters, the cytocentrifuge would drive cells less than $8.5\ \mu\text{m}$ in diameter into the first $5\text{--}15\ \mu\text{m}$ of the filter surface allowing their observation.

Leukemic myeloblasts were also placed between nucleopore filters of various pore diameters. Since they were sandwiched between the two filters, there was no cell loss from shedding. Nevertheless, egress did not occur (Fig. 6). Indeed, myeloblasts did not show any evidence of attempting to traverse the filters even when observed for up to 24 h. Occasionally, myeloblasts could

be seen abutting pore orifices and deforming them suggesting that marrow leukemic blast cells could, especially if in high density, exert pressure effects on a barrier wall.

DISCUSSION

Our observations support the possibility that control of marrow release could be determined by the interaction of marrow sinusoidal wall porosity and the development of cellular biophysical characteristics favoring cell egress during cell maturation (Fig. 7).

Electron microscopy of marrow has demonstrated that the marrow sinusoid has a basic trilaminar structure composed of an adventitial cell facing the hematopoietic cord, a basement membrane-like structure and an endothelial cell lining the marrow sinusoid (1). Pores are present in endothelial cells. The basement membrane may be interrupted and adventitial cells may be absent (1). Controversy exists as to whether pores are an intrinsic feature of marrow wall or whether pores develop intermittently, perhaps as a result of abluminal pressure produced by abutting cells in the hematopoietic cords (2).

Studies of maturing granulocytes have indicated that cytoplasmic maturation which favors adhesiveness, mo-

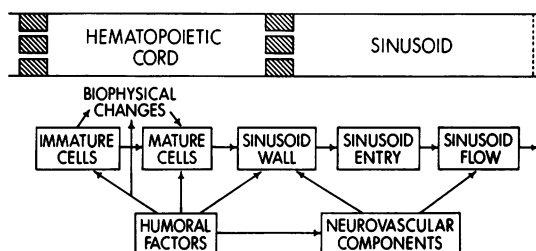


FIGURE 7 A schematic diagram of the possible factors involved in controlling release of marrow granulocytes. The central relationship of the hematopoietic cord to the sinusoid is shown. In the hematopoietic cord maturation of cells adapts them for egress (6, 7). The number of maturing cells and the rate of maturation may be governed in part by humoral factors (granulopoietins) and chalone (granulosuppressins [32]). The marrow barrier or sinusoid wall also appears to be a dynamic structure, and its porosity may be influenced by humoral agents (4) as well as cell crowding (30) particularly by rigid leukemic blast cells (31). The role of modulation of sinusoidal delivery has not been defined, although evidence of extensive neurovascular networks (25) suggests the possibility of neural or humoral control of sinusoidal flow into the efferent circulation of bone. The latter may be particularly important for erythrocyte delivery.

tility, and deformability occurs at the late myelocyte stage (6, 7). These changes provide the biophysical prerequisites for the negotiation of the restrictive barrier containing pores with diameters smaller than that of the cell. Alterations in nuclear shape and deformability may also contribute to the ability to negotiate a small diameter orifice.

However, these two factors alone cannot explain the supply and demand system that regulates marrow cell delivery during periods of increased or decreased need. One important modifying factor could be the superimposition of a humoral agent to foster cell release (e.g., granulocyte-releasing factor). Evidence for such a factor has accumulated in several species (9, 11, 16-18) including man (10). In an effort to simulate this additional factor a chemoattractant agent was added to the system. The use of an attractant demonstrated that immature human cells do not respond to complement-activated chemotaxis. The inability of human immature granulocytes to respond to an attractant in our system could relate to their poorly developed apparatus for motility and their inability to traverse narrow pore diameters. Kass and DeBruyn observed an unresponsiveness of rabbit immature granulocytes to chemotactic agents derived from bacterial sources in a system that did not involve restrictive barriers and that was independent of rate of migration (19). These observations suggest that specific surface receptors develop, probably at the late (small) myelocyte stage which allow response to complement-dependent chemoattractants. We do not know whether a granulocyte-releasing factor is

closely analogous in its action; however, it is possible that development of the responsiveness to such a humoral agent occurs at the later stages of granulocyte development. This is in keeping with the observation that several important changes in the cell periphery occur during late granulocyte development (7). In vivo, it is possible that a cell-releasing factor could operate by increasing the rate of cell egress of mature cells by acting as a chemoattractant. Also, our results demonstrate that a humoral factor can accelerate egress of mature granulocytes in direct relationship to barrier pore size.

The factor responsible for accelerating granulocyte release may also enhance multiplication and rate of maturation of granulocytes, analogous to the apparent dual effect of erythropoietin on erythropoiesis (20) and on the release of reticulocytes (11, 21, 22). Alternatively, at least two humoral agents may be involved in sustained leukocytosis, one fostering accelerated release and one enhancing proliferation (13). The former could act on receptors at the mature granulocyte periphery to accelerate movement, or on the marrow barrier to increase pore size (4), or both, whereas, the latter could act on nuclear receptors of immature hematopoietic cells to enhance cell mitosis and maturation rate.

A fourth factor in marrow release may be the regulation of microcirculatory (sinusoidal) flow. As yet, this aspect of the marrow circulation has been difficult to approach experimentally. Cells entering the sinusoids from hematopoietic cords could be retained in the marrow if smooth muscle sphincters governed sinusoid delivery. The role of sphincters in control of microcirculatory flow has been reappraised recently (23). Although such sphincters have not thus far been observed in marrow, Schwann cells (24) and extensive neurovascular networks (25) are present, indicating that the anatomic and physiologic prerequisites for such control exist. Sphincters have been described in fetal rat liver, where islands of erythropoiesis are present in sinusoids (26). These sphincters appear to be sensitive to erythropoietin indicating that in the fetal rat, a single humoral agent may be responsible for increased erythroid proliferation and control of delivery of cells. This dual effect of erythropoietin has also been suggested by studies in experimental hemolytic anemia in rats, in which increased sinusoidal wall pore size has been observed during periods of increased erythrocyte need (4), although the effect of erythropoietin on marrow stroma has not been established directly.

The control of release of erythroid and granulocytic cells may be different since the erythrocyte is not known to respond to chemoattractants and has only an abortive motile apparatus at the marrow reticulocyte stage. The possible importance of the development of the ability to

traverse small pores during erythroid maturation has been shown (8) and this may be related to the enucleation of the erythrocyte. However, the modulating factor in erythroid cell delivery may be the control that exists over the delivery of sinusoid contents.

Control over release of platelets has not been postulated. Increased production appears to be the primary mechanism to meet increased need. This would be in keeping with the inability of the porous sinusoid wall to retain particles the size of platelets with a mean diameter of 2.3 μm .

The system is imperfect, and some immature cells escape probably because they abut larger diameter openings ($> 8 \mu\text{m}$). Because of the biophysical properties of immature cells, they are probably trapped in spleen, lung, or other locations and may complete maturation in those sites.

The inability of leukemic blast cells to traverse small diameter orifices and to respond to a chemotactic agent is probably not a reflection of leukemia per se rather an index of immaturity. Leukemic blast cells may be capable of developing into mature granulocytes (27) and monocytes (28). Studies of mature granulocytes in leukemia patients would be required to determine if a defect in chemotaxis exists in such cells. Evidence has already been gathered which indicates that mature neutrophils in some subjects with leukemia may have reduced adhesiveness (28) and subnormal capacity to enter the tissue compartment (29). The inability of leukemic blast cells to traverse small diameter pores suggests that the egress of leukemic cells may be dependent on (a) cell size (smaller cells more likely to escape), (b) enlargement of marrow pores which may occur from albuminal pressure in leukemia marrow by dense populations of blast cells, or (c) destruction of stromal architecture and the loss of the normal relationships of hematopoietic cords to marrow sinusoids. The latter change has been described in a recent study of marrow in rat chloroleukemia (30). Studies in our laboratory indicate that leukemic blast cells do not traverse pores of diameter smaller than that of the cell unless a high pressure gradient is applied (31). Like normal myeloblasts and progranulocytes, leukemic blast cells may not respond to chemoattractants because of an inability to migrate and deform rather than an absence of surface receptors.

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