CHEMOTAXIS OF MONONUCLEAR CELLS*

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The appearance of mononuclear cells in inflammatory exudates has never been adequately explained, possibly because factors leading to the selective migration of mononuclear cells have rarely been described. Differences in migration rates for neutrophils and mononuclear cells (the former having a faster rate) might explain why these two cell types do not appear simultaneously during development of an inflammatory reaction (1). By this reasoning, the factors that control the efflux of neutrophils from vessels would exert an identical effect on monouclear cells. In his review Harris (2) states "... the polymorphonuclear leukocyte and the monocyte reacted chemotactically to the same stimuli. No substance was found which was chemotactic for one cell without being chemotactic for the other." The recent report of Keller and Sorkin (3), who used the micropore-filter technique (4) for the study of chemotaxis, supports the conclusion of Harris. Nevertheless, if a factor specifically chemotactic for mononuclear cells were to be found, this might be relevant to an understanding of the inflammatory response.

There is some indirect evidence relating the accumulation of mononuclear cells to a prior influx of neutrophils, since the absence of circulating neutrophils in large part precludes the appearance of mononuclear cells in experimentally induced inflammatory reactions (5). Restoration of circulating neutrophils is reported to correct this deficiency of mononuclear cells. In addition to this evidence, markedly diminished accumulations of mononuclear cells in inflammatory exudates have been found in association with the clinical condition of cyclic neutropenia (6). In this syndrome when the number of circulating neutrophils diminishes, few mononuclear cells migrate into acute inflammatory lesions of the dermis (studied by the Rebuck skin-window technique [7]), whereas no such defect is seen when circulating neutrophils return to normal levels. These data have indicated some relationship between the influx of neutrophils in an inflammatory exudate and the subsequent appearance of mononuclear cells. The importance of an understanding of the factors re-

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sponsible for the accumulation of mononuclear cells in inflammatory infiltrates can hardly be understated, since these cells comprise a substantial portion of leukocytes in many inflammatory exudates.

This paper deals with a study of factors chemotactic for mononuclear cells. The technique for the study of chemotaxis of mononuclear cells employed the same type of chamber used in studies of chemotaxis of neutrophils (4, 8) with one important exception—the filters had larger pore sizes (3). We have defined two chemotactic factors to which both neutrophils and mononuclear cells respond and two chemotactic factors specific for mononuclear cells alone. One of these latter factors was obtained from lysates of neutrophils, indicating a possible functional relationship between neutrophils and mononuclear cells. An appreciation of these various factors may have some relevance to an understanding of the inflammatory response.

Materials and Methods[1]

Chemotaxis Chambers.—Stainless steel chambers, each separated into two compartments by a micropore filter, were the same as those previously employed in the study of neutrophil chemotaxis (8). An important difference was the larger pore size of the micropore filter. As recently reported by Keller and Sorkin (3), filters, type SWMP 02500, pore size 5 μ (Millipore Filter Corp., Bedford, Mass.), were used for studies on chemotaxis of mononuclear cells. The suspending medium and the techniques for assembling, handling, and processing the filters were the same as those used for neutrophils (8). In most experiments cells were suspended in 10% homologous (rabbit) serum. In experiments in which organophosphonate esters were used, cells were suspended in 0.5% ovalbumin (9). Incubation times for chambers were 5 hr unless otherwise indicated. In quantitating the chemotactic responses only those cells (in five random high-power fields) that had migrated completely through the interstices of filters to form a monolayer on its opposite side were counted. This method of counting cells is selective, in that migrating cells that have not reached the opposite side of the filter are *not* counted. This contrasts with our studies on chemotaxis of neutrophils in which *all* migrating cells were counted (8). As will be shown in a later section, the same relative value for chemotaxis is obtained, regardless of whether all migrating cells are counted or only those that have migrated completely through the filter. The latter counting procedure is used because it is easier and affords greater accuracy.

Cells.—All mononuclear cells except alveolar macrophages were obtained by inducing peritoneal exudates in rabbits (10). The term "mononuclear cell" does not imply a specific cell type but, rather, describes a general category of cells. Most of the cells in the exudates consist of mononucleated cells having abundant cytoplasm. The nuclei occasionally may contain a large cleft, but they are not multilobed. Generally, approximately 80% of the cells in an exudate can be classified as mononuclear cells, while 15% of the remaining cells are neutrophils and 5% lymphocytes. To obtain exudates, a solution of 50 ml of sterile mineral oil, USP, light, white (Henry B. Gilpin Co., Baltimore, Md.), or 100 ml of 1% hydrolyzed starch suspension (Connaught Medical Research Laboratories, Toronto, Canada) was boiled in saline and injected intraperitoneally into adult male New Zealand rabbits. 4 days later the exudates were harvested by abdominal paracentesis and suspended in sterile saline. Cell pellets were re-

[1] The "Principles of Laboratory Animal Care" as promulgated by the National Society for Medical Research were observed during this study.

covered by centrifugation at low speed (approximately 500 *g* for 10 min) and then resuspended in appropriate medium.

Alveolar Macrophages.—Alveolar macrophages were obtained by washout of the pulmonary tree with saline, according to the method of Cohn and Wiener (10), and morphologically the alveolar macrophages appeared similar to cells described by them.

In the experiments utilizing lysates of neutrophils, the cells were obtained in a glycogen-induced peritoneal exudate in rabbits (8, 11). At least 98% of the cells produced by this method were neutrophils. Lysates were obtained by freeze-thawing five times and the removal of cell debris by centrifugation.

Chemotactic Preparations.—When rabbit serum was treated with immune complexes, the reagents consisted of bovine serum albumin (BSA) and antibody-rich IgG (rabbit) obtained by ion exchange chromatography as previously described (12). 100 μg antibody N with antigen at equivalence was added to each 0.1 ml of fresh rabbit serum and incubated for 30 min before dilution and addition to lower compartments of chambers. The preparation containing pneumococcal (Type I) chemotactic factor, which was active for neutrophils, was the bacteria-free culture filtrate in medium 199 described elsewhere (13). Serum treated with plasmin to generate the chemotactically active fragment of the third component of complement (14, 15) was prepared by adding 1 mg plasminogen and 2 mg streptokinase (Dr. Fletcher Taylor, Jr., University of Pennsylvania) to each 1.0 ml fresh serum and incubating for 1 hr at 37°C. This preparation is chemotactically active for neutrophils (14). Preparations rich in the activated trimolecular complex, consisting of the fifth, sixth, and seventh components of complement, C′(5, 6, 7)a, were obtained by ion exchange chromatography. This material is chemotactically active for neutrophils also, and its preparation is described in detail elsewhere (8).

Complement-Deficient Serums.—Naturally C′6 deficient rabbit serum was kindly supplied by Dr. Klaus Rother, Max Planck Institut fur Immunbiologie, Freiburg, Germany. Artificial depletion of complement in rabbit serum was accomplished by treatment with potassium thiocyanate (Fisher Scientific Company) exactly according to the procedure of Dalmasso and Müller-Eberhard (16). After treatment the KSCN was removed by extensive dialysis. Such serum, whether treated with 0.1 M or 1.0 M KSCN, was complement-deficient according to three criteria: (*a*) complete loss of hemolytic activity, assessed by the addition of sensitized erythrocytes (EA); (*b*) inability to make up intermediate complexes consisting of the first three reacting complement components EAC′1a, 4, 2a as determined by the fact that EA incubated with KSCN-treated serum, washed, and then added to normal rabbit serum with ethylenediaminetetraacetate (EDTA) failed to undergo lysis (see Ward and Cochrane (12) for details); (*c*) gross alteration in the immunoelectrophoretic behavior of third component (C′3 or β-1C globulin) in KSCN treated serum. These findings imply that several complement components have been altered by the KSCN, including at least two of the first four reacting components.

Preparative Electrophoresis and Ultracentrifugation.—Serum treated with immune complexes was separated into various fractions by electrophoresis in Pevikon (17), employing a phosphate buffer, pH 7.3, ionic strength 0.05. Ultracentrifugal separation of the treated serum or neutrophil lysate was accomplished in a 7.5–35% sucrose gradient in phosphate buffer (as above), using a B-60 ultracentrifuge with an SB-405 swinging bucket rotor (International Equipment Co., Needman Heights, Mass.). Centrifugation was carried out at 55,000 rpm for 16 hr at 4°C.

Phosphonate Esters.—These compounds (*p*-nitrophenyl ethoxy alkyl phosphonates) which specifically and irreversibly react with esterases containing serine in or near the active site (18) were kindly provided by Dr. Elmer L. Becker, Walter Reed Army Institute of Research. The phosphonates were used in the same manner as described for the neutrophil chemotaxic system in which cell-dependent inhibition of chemotaxis was described (9, 19).

Cell-Dependent Inhibition of Chemotaxis.—In this procedure, cells were incubated with ap-

propriate phosphonate or control solution (acetone 1:200 in medium 199) for 2 hr at room temperature and then washed, resuspended in 0.5% ovalbumin, and tested for chemotactic responsiveness either to serum activated by incubation with an immune complex or to the pneumococcal chemotactic factor. The per cent of inhibition of chemotactic responsiveness was then calculated. Inhibition profiles, i.e., the ability of various types of phosphonates within three classes (alkyl, chloralkyl, and phenylalkyl) to cause cell-dependent inhibition of chemotaxis, were determined and compared with data similarly obtained with neutrophils (9).

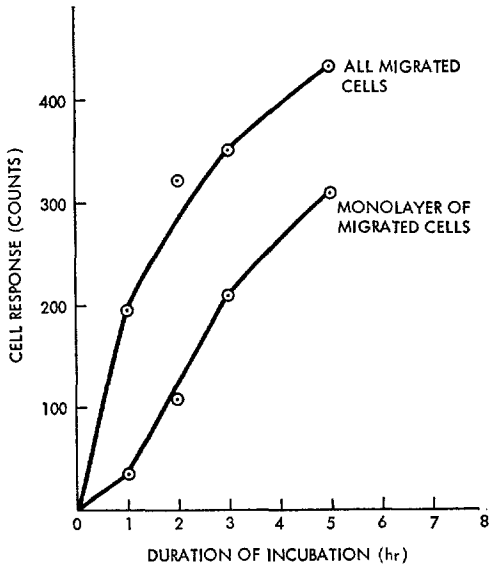

FIG. 1. Time course in the chemotactic response of mononuclear cells. The two graphs represent counts of cells in five high-powered fields. In one case all cells moving through the filter have been counted, whereas in the other, only cells that have moved completely through the filter and are present on the opposite (bottom) side have been counted. The curves are relatively parallel.

RESULTS

Time Course of the Chemotactic Response.—Chambers were sampled at various intervals in order to assess the effect of time on the course of the chemotactic response of mononuclear cells obtained from an oil-induced exudate. The source of chemotactic factor was rabbit serum treated with immune complexes. Two categories of cells were counted: (*a*) those that had migrated completely through the filter, forming a cell monolayer on the opposite (lower) surface, and (*b*) all cells in the process of migration. The data in Fig. 1 indicate that after a 5-hr interval (when the experiment was usually terminated), the reaction had begun to taper off although it was apparently not yet complete. At 3- and 5-hr intervals more than half the cells in the process of migration had reached the

opposite (bottom) side of the filter. Because of the relative ease and greater accuracy in counting only cells on the opposite side of the filter, this was the method used to assess chemotaxis unless otherwise indicated. In Fig. 6 filters from two different chambers are compared. Focusing on the upper monolayer containing most of the cells originally added to the upper compartment, the sheet of mononuclear cells is clearly seen (frames a and c). When the focus is shifted to the opposite (bottom) surface of the filter, cells which have migrated can be seen clearly, although nuclear and cytoplasmic boundaries are not well

TABLE I

Requirements for Generation of Mononuclear Cell Chemotactic Factor and Positional Effect of Factor in Chemotaxis

Experiment	Material tested	Chemotactic activity*
A	Serum‡	24
	Serum and immune complex	120
	Heated serum (56°C, 30 min) and immune complex	21
	Immune complex in medium 199	18
	C'6-deficient serum and immune complex	98
	0.1 M KSCN-treated serum and immune complex	128
B§	Serum treated with immune complex, in lower compartment	105
	Serum treated with immune complex, in upper compartment	5
	Serum treated with immune complex, in both compartments	18

* Cells were suspended in 10% normal rabbit serum.

‡ Rabbit serum used throughout these studies.

§ The immune complex was removed from serum by centrifugation after incubation at 37°C for 1 hr.

defined (Fig. 6, frames b and d). Electronmicroscope studies in conjunction with Dr. Howard Scalzi revealed the presence of typical mononuclear cells migrating through interstices of filters (Fig. 7).

Requirements for Generation of Chemotactic Factor.—In order to define the factors essential for generation of substances in serum which are chemotactic for mononuclear cells (obtained from a mineral oil exudate), several preparations of rabbit serum were employed. The addition of an immune precipitate consisting of rabbit anti-BSA and antigen at equivalence to fresh rabbit serum resulted in the appearance of chemotactic activity for mononuclear cells (Table I). Preheating the serum destroyed its capacity to support generation of the chemotactic factor. The fact that the immune complex in the absence of serum was chemotactically inactive confirmed the need for serum factors in the generation of the chemotactic substance (Table I). It was found, however,

that with C'6-deficient rabbit serum and with serum artifically rendered deficient in at least two of the complement components reacting early in the sequence (see Materials and Methods section dealing with KSCN-treated serum), there was little difficulty in generating chemotactic activity. This provides sharp contrast with the requirements for generation of C'(5, 6, 7)a, chemotactic factor for neutrophils, from the complement system in which seven of the nine complement components are required (8, 20). The complement-associated factor chemotactic for neutrophils cannot be generated in such complement-deficient sera. These data indicate that the chemotactic factor for neutrophils and the chemotactic factor for mononuclear cells can be differentiated on this basis of requirements for generation. In experiment B

TABLE II

Effectiveness of KSCN-Treated Rabbit Serum in Generation of Mononuclear Cell Chemotactic Factor

Material tested	Chemotactic activity*
Serum	25
Serum and immune complex	240
0.1 M KSCN-treated serum	20
0.1 M KSCN-treated serum and immune complex	250
1.0 M KSCN-treated serum	25
1.0 M KSCN-treated serum and immune complex	310

* Cells were suspended in 10% normal rabbit serum.

(Table I) it was demonstrated that serum activated by the addition of immune complexes satisfied a cardinal requirement for a chemotactic factor—only a factor added at a distance from cells (i.e. in the lower compartment) was active. Addition of the factor directly to the upper compartment, which contained the cell suspension, was ineffective in inducing cell migration, just as it was in the case of neutrophils (20).

To further define the requirements for generation of the chemotactic factor for mononuclear cells, several preparations of KSCN-treated serum were used, varying only in the original concentration of KSCN used to inactivate the complement components (Table II). It seemed important to test KSCN-treated serum that had not been incubated with immune complexes to determine whether the serum per se was chemotactically active, particularly since such treated serum contains several altered complement components (16). This serum per se was not chemotactically active (Table II). Addition of immune complexes, however, resulted in the appearance of chemotactic activity. It was interesting to note that serum treated with the higher concentration of KSCN had an enhanced ability to generate chemotactic activity (count of 310 vs. 250).

This finding has been confirmed in two other experiments. In all cases, the serum was hemolytically inactive for complement activity.

Comparison of Neutrophils and Mononuclear Cells in Chemotaxis.—Rabbit neutrophils obtained by glycogen-induced peritoneal exudate and mononuclear cells induced by the injection of either mineral oil or starch were tested for chemotactic activity against various preparations, several of which were known to be chemotactic for neutrophils. As anticipated, neutrophils responded to serum that had been incubated with immune complexes or with mixtures of

TABLE III

Reactivity of Various Peritoneal Cells to Chemotactic Factors

Preparation tested	Chemotactic activity*			
	Neutrophils	Mononuclear cells		
		Mineral oil	Starch	Alveolar macrophages
Serum	0	35	4	8
Serum and immune complex	401	217	9	15
Serum and immune complex, heated to 56°C, 1 hr		201		
1.0 ml rabbit serum, 1 mg streptokinase, and 0.5 mg plasminogen‡	257	203	198	17
Pneumococcal chemotactic factor (100 μl)	278	113	113	28
Pneumococcal factor, dialyzed 18 hr		14	16	
Serum treated with 0.1 M KSCN		14		
KSCN-treated serum and immune complexes	25	221		
Medium 199	0	42	14	9

* All cells were suspended in 10% normal rabbit serum.

‡ 0.1 ml treated serum used for chemotactic testing.

streptokinase and plasminogen (Table III). A culture filtrate from *Diplococcus pneumoniae*, Type I, was also active. *Mononuclear cells* obtained from the mineral oil-induced exudate were active to the same two serum preparations, and activity—once generated in serum by the addition of immune complexes—was resistant to the effects of heating thereafter. The bacterial chemotactic factor was also active for mononuclear cells, and this activity, as with findings with the factor that is active for neutrophils (13, 21), disappeared during dialysis (Table III). Keller and Sorkin reported similar findings (21). Serum treated with potassium thiocyanate was unable to support generation of chemotactic activity for neutrophils after immune complexes were added, but considerable chemotactic activity for mononuclear cells (from the mineral oil exudate) was generated. This again stresses the difference in requirements for generation of chemotactic factors for neutrophils and mononuclear cells.

Mononuclear cells from the starch-induced exudate behaved similarly to those obtained by injecting mineral oil, with one important exception: Serum treated with immune complexes was not chemotactically active (Table III). This finding, confirmed in three subsequent experiments, indicates a functional difference between mineral oil-induced and starch-induced mononuclear cells.

Alveolar macrophages, which also comprise a category of mononuclear cells, were found to be relatively inactive in chemotaxis when tested for responsiveness to three factors known to be very active for mononuclear cells from a

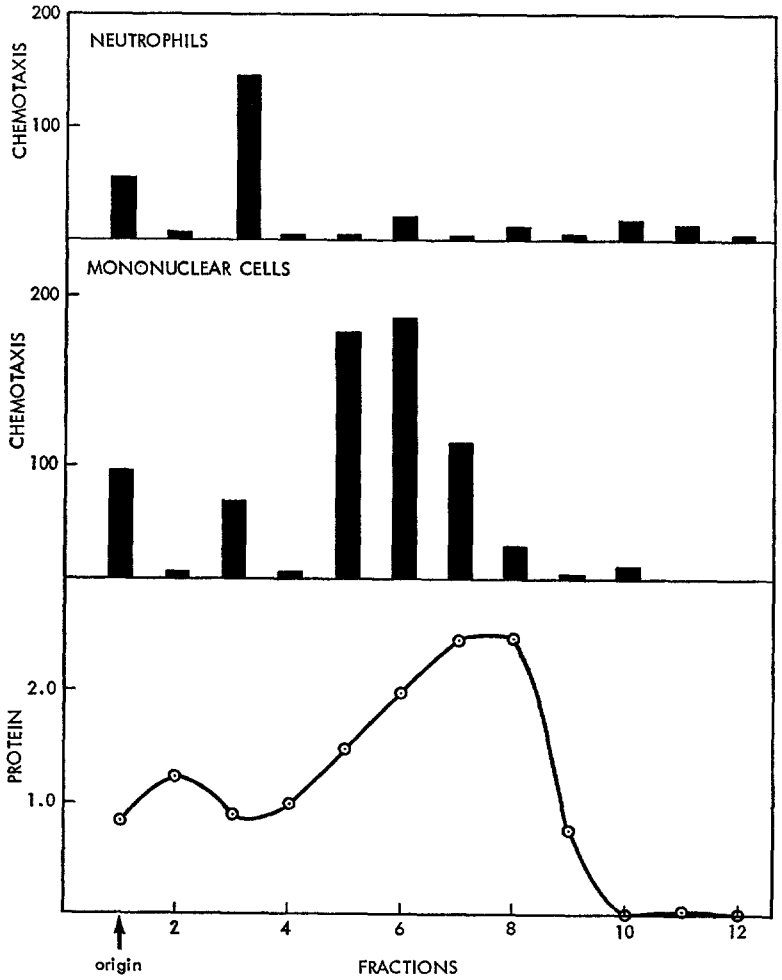

FIG. 2. Analysis for chemotactic activity in electrophoretic fractions of normal rabbit serum (NRS) treated with immune complexes. The chemotactic factor for neutrophils is distinctly less rapid in its migration (upper frame) than that for mononuclear cells (middle frame). Anode is to the right.

mineral oil exudate (Table III). Alveolar macrophages responded very poorly to serum treated with either immune complexes or plasminogen-streptokinase. A slightly greater response to the bacterial chemotactic factor was noted, and this has been the general trend in three other experiments. These data indicate that alveolar macrophages respond very weakly to chemotactic factors (at least to factors which are active for other mononuclear cells).

Physical-Chemical Differences in Chemotactic Factors for Neutrophils and Mononuclear Cells in Serum.—Since the chemotactic factor for neutrophils in serum appearing after addition of immune complexes requires complement for its generation, whereas the mononuclear cell chemotactic factor does not require complement (Tables I, II, and III), attempts were undertaken to define in physical-chemical terms these two chemotactic factors. Normal rabbit serum (NRS) treated with immune complexes was separated into electrophoretic fractions, and the results are shown in Fig. 2. The protein profile of these fractions reveals two main areas (lower frame, Fig. 2). Chemotactic activity for neutrophils was confined primarily to fraction number 3, although some activity, presumably in aggregated protein, was also found at the origin (upper frame, Fig. 2). All other zones failed to show significant chemotactic activity for neutrophils. In contrast to this, the activity for mononuclear cells was largely confined to fractions 5, 6, and 7 (middle frame, Fig. 2), although some activity was present at the origin and a small amount of activity was also present in fraction 3, the same fraction active for neutrophils. In general, however, the chemotactic factor for mononuclear cells could be clearly distinguished from that of neutrophils by its more rapid migration in the electrical field.

Another way of differentiating the two chemotactic factors was found by ultracentrifugation in sucrose density gradients. As shown in Fig. 3 (frame a), the chemotactic activity for mononuclear cells was confined to the upper portion of the gradient (fractions 11 and 13), whereas the chemotactic factor for neutrophils, consisting of C′(5, 6, 7)a, sedimented appreciably faster and was found in fractions 7, 9 and 11 (Fig. 3, frame b). This latter factor generally occupied the lower half of the region in the gradient containing C′6 activity, and this is in accord with previous findings in which the position of C′(5, 6, 7)a was determined (8, 20). The mononuclear cell chemotactic factor had a sedimentation velocity distinctly slower than that of bovine gamma globulin, which was added separately as a marker protein. These findings indicate that two different chemotactic factors for leukocytes are generated in serum by the addition of immune complexes. One factor is active for neutrophils, and the other is active for mononuclear cells. Not only do the factors differ in their requirements for generation, but they can also be distinguished on the basis of physical-chemical differences (velocities of sedimentation and electrical charges).

A Neutrophil-Associated Factor that is Chemotactic for Mononuclear Cells.—

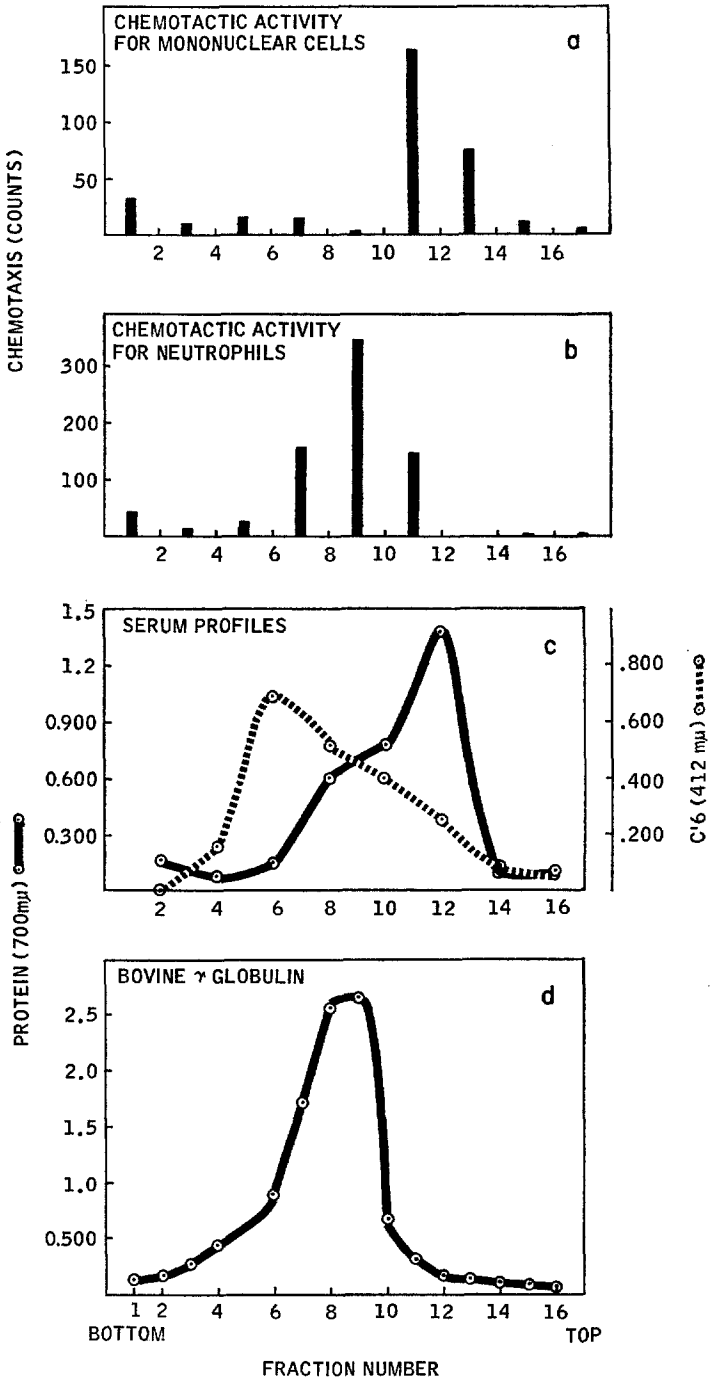

FIG. 3. Analysis for chemotactic activity in ultracentrifugal fractions of serum treated with immune complexes. The chemotactic factor for mononuclear cells (frame *a*) sediments much more slowly than the factor chemotactic for neutrophils (frame *b*).

Mononuclear cells obtained from a mineral oil-induced exudate were responsive, as anticipated, to serum treated with immune complexes or plasminogen-streptokinase and to the bacterial chemotactic factor (Table IV). In addition, a potent factor in lysates of rabbit neutrophils was chemotactically active for mononuclear cells (Table IV, experiment A). This factor was somewhat heat labile but was not appreciably affected by dialysis in medium 199 (Table IV, experiment B). Mononuclear cells did not respond chemotactically to any

TABLE IV

Mononuclear Cell Chemotactic Activity in Lysates from Neutrophils

Cells tested	Material tested	Chemotactic activity
Mononuclear cells		
Experiment A	Normal rabbit serum (10%)	24
	Serum and immune complex	228
	0.1 ml serum and 0.1 mg plasminogen + 0.05 mg streptokinase	135
	Pneumococcal factor (100 μl)	258
	Lysate of 1×10^7 neutrophils	500
	Lysate of 5×10^7 mononuclear cells	15
	Medium 199	35
Experiment B	Neutrophil lysate (1×10^7 cells), untreated	95
	Neutrophil lysate (1×10^7 cells), heated (56°C, 1 hr)	66
	Neutrophil lysate (1×10^7 cells), dialyzed (18 hr)	89
	Medium 199	13
Neutrophils		
	Normal rabbit serum	2
	Serum and immune complex	255
	Neutrophil lysate (1×10^7 cells)	18
	Mononuclear lysate (5×10^7 cells)	0
	Medium 199	0

factors contained within mononuclear cells. When the same cell lysates were tested in the neutrophil chemotactic system, no significant activity was found either in lysates of neutrophils or mononuclear cells. These findings indicate that mononuclear cells can respond chemotactically to a factor in neutrophils, whereas the converse is not true.

The chemotactic factor for mononuclear cells in lysates of neutrophils was analyzed by ultracentrifugation in a sucrose-density gradient. While a sharp resolution was not obtained, probably because of the application of an excessive amount of factor, activity was confined to the upper portion of the gradient (Fig. 4), suggesting the presence of material of low molecular weight. The

relative ionic charge of this material is not yet known, although we have found chemotactic activity for mononuclear cells in highly purified cationic peptides of rabbit neutrophils, kindly supplied by Dr. N. Ranadive and Dr. C. G. Cochrane, La Jolla, California.

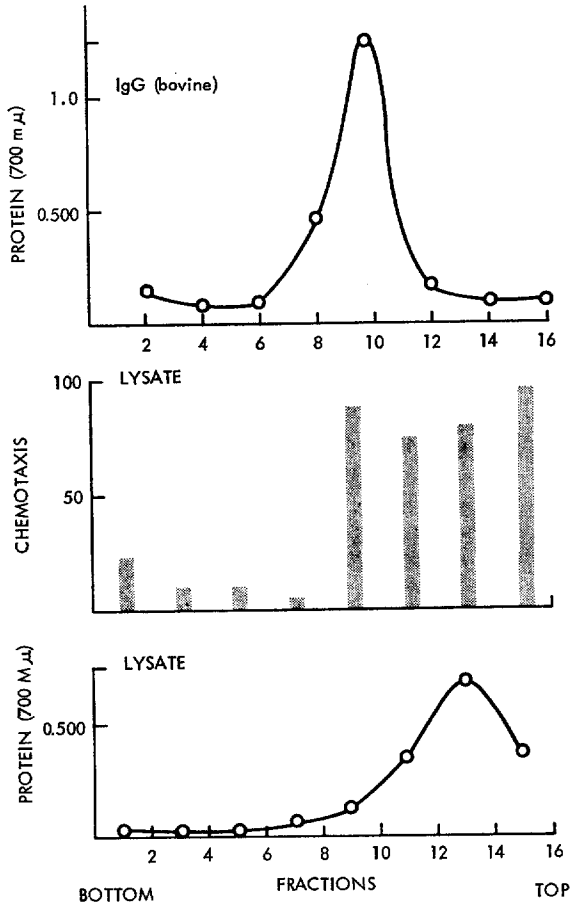

FIG. 4. Analysis by ultracentrifugation in sucrose-density gradient of mononuclear cell chemotactic factor in lysates from neutrophils. The chemotactic activity is confined to the upper portion of the gradient (middle frame).

Differences between Neutrophils and Mononuclear Cells in Susceptibility to Phosphonate-Induced Inhibition of Chemotaxis.—Phosphonate esters irreversibly inhibit chemotaxis in neutrophils by blocking either of two esterase systems: the so-called "activated esterase", which appears similar to an acetylase (9, 19), and the "activatable" esterase (proesterase), which has an affinity for aromatic acid compounds (22). Since it was not known whether mononuclear cells

contain esterases with serine in or near the active center, cells were preincubated with phosphonate esters and then tested for chemotactic activity. The chemotactic factors used were serum preincubated with immune complexes or the soluble pneumococcal factor obtained from pneumococcal culture filtrates.

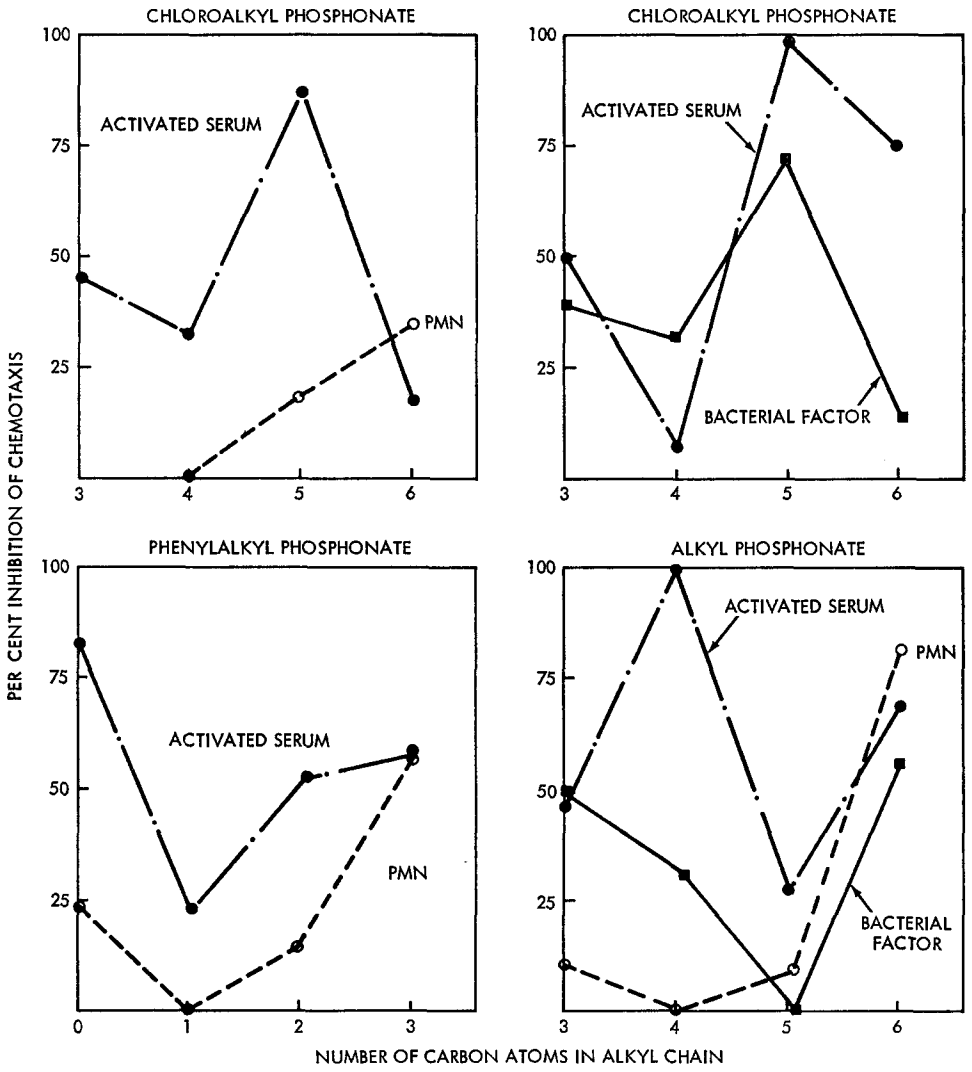

FIG. 5. Inhibition profiles of the chemotaxis of mononuclear cells obtained with the use of several series of phosphonate compounds. "Cell-dependent inhibition" as illustrated here is the irreversible effect of treatment of cells prior to chemotactic testing. Inhibition profiles for neutrophils are different from those for mononuclear cells. Interrupted lines represent profiles for neutrophils (PMN).

Homologous series of phosphonate esters were used in order to obtain "inhibition profiles" of chemotaxis.

In Fig. 5 inhibition profiles are plotted according to the number of carbons in the alkyl, chloroalkyl, or phenylalkyl side chains. As is first evident, several phosphonates at 5×10^{-4} M were very effective inhibitors, indicating that there is indeed a serine esterase in mononuclear cells that is necessary for chemotactic responsiveness of the cells. For comparison, cell-dependent inhibition profiles for neutrophils are included in Fig. 5. These data were obtained from Fig. 6 of an earlier study (9). Several conclusions can be drawn from these findings: (*a*) The most effective inhibitors of chemotaxis of mononuclear cells are the 5-chloropentyl phosphonate, the phenyl phosphonate, and the butyl phosphonate. (*b*) The inhibition profiles for mononuclear cells responding chemotactically to serum pretreated with immune complexes are the same as the profiles of similar cells responding to the bacterial chemotactic factor. This implies that a similar, or identical, serine esterase in the mononuclear cell is involved in both chemotactic responses. (*c*) The inhibition profiles for mononuclear cells in chemotaxis are different from the profiles for neutrophils. While both cell types are similarly affected by the phenyl phosphonates (Fig. 5), there are rather striking differences when the profiles of the chloroalkyl and the alkyl series are compared. If the profiles from the five carbon to the six carbon of chloroalkyl phosphonates are compared, an increased inhibition of neutrophils is found, whereas the inhibition of chemotaxis of mononuclear cells falls. The same divergency is seen with the alkyl series: when trends are compared for inhibition by the four carbon to the five carbon compounds, there is a slight rise in inhibition of neutrophils by the five carbon compound, whereas an abrupt fall in inhibition of mononuclear cells occurs. These findings indicate that mononuclear cells contain a serine esterase that is necessary for chemotactic responsiveness, but the enzyme is different from the analogous esterase in the neutrophil, as defined by this technique of cell-dependent inhibition.

DISCUSSION

Perhaps the most interesting aspect of this study is the finding of two factors that are selectively chemotactic for mononuclear cells. The chemotactic factor for mononuclear cells appearing in serum after addition of immune complexes can be contrasted with C′(5, 6, 7)a in the following ways: Complement is apparently not required for generation of the factor; the chemotactic factor bears a relatively acidic charge compared to C′(5, 6, 7)a; and it sediments in the ultracentrifuge at a slightly slower rate. The most contrasting feature of all, however, is the specificity of the biologic activity for mononuclear cells. There appear to be very few, if any, reports describing factors selectively chemotactic for these cells.

In the Arthus reaction mononuclear cells usually appear after the initial in-

terval, which features the rapid accumulation of neutrophilic granulocytes in and around vessels containing deposits of antigen, antibody, and complement (12, 23). After 5–6 hr, mononuclear cells, consisting of monocytes and lymphocytes, begin to accumulate in the exudate. As Cochrane has pointed out in his recent review of the Arthus reaction, there is a gradual transformation to the nongranulocytic series (24). The delay in appearance of mononuclear cells could well result from the slower rate of migration of these cells compared to granulocytes. This can be readily appreciated by differences in the time courses of chemotaxis for neutrophils and mononuclear cells. The neutrophil responds within a 90-min interval (9), whereas the mononuclear cell has not yet completed its response after 5 hr (Fig. 1). On the other hand, the findings reported in this paper indicate the equally plausible explanation that the appearance of the mononuclear cell may result from the participation of a selective chemotactic factor for this type of cell. In interaction between immune complexes and serum proteins in tissues, both the chemotactic factor for neutrophils, C′(5, 6, 7)a, and the chemotactic factor for mononuclear cells could be generated. It is not possible to determine which factor, if either, may be operative in the regulation of accumulations of leukocytes in immunologically determined inflammatory responses. When bacterial chemotactic factors or the plasmin-generated chemotactic factor is produced in tissues, it is possible that both neutrophils and mononuclear cells respond chemotactically as they do in vitro (13, 15), but that they do so at different rates characteristic of the leukocyte rather than of the chemotactic factor.

The factor in the neutrophil that is chemotactically active for the mononuclear cell is of exceptional interest. The presence of such a factor in the granulocyte implies that, in the course of the inflammatory exudate, the neutrophil carries into the tissue site a substance that, upon release, can modify the reaction by attracting mononuclear cells from the circulation. Thus, the usual transition in the type of leukocyte in the exudate would be predetermined the moment the granulocyte arrived at its extravascular site. The presence of this chemotactic factor in the neutrophil agrees with the studies on clinical and experimental inflammation, in which it was found that the appearance of mononuclear cells in exudates must be preceded by the presence of neutrophils. The story is not that simple, however, because in pneumococcal lobar pneumonia massive numbers of neutrophils appear in alveolar spaces, only to be cleared within 48 hr without any apparent transition from neutrophils to mononuclear cells. Whatever the explanation for this event, it is obvious that the mere presence of a neutrophil in an exudate does not guarantee subsequent appearance of the mononuclear cell.

One of the problems in the study of leukocytic chemotaxis in which the modified Boyden chamber is used concerns the identification of the cell that has migrated into or through the micropore filter. In contrast to the report by Keller

and Sorkin (3), we found it difficult to positively identify the type of responding cell by light microscopy with the usual techniques for preparation and staining of filters. This is partly because of the poor cytoplasmic detail of cells. (The filter takes the cytoplasmic stains more avidly than the leukocytes.) In addition, cells appear to become rather distorted in the process of migration through the filter. Even when electron microscopic technique was employed, we sometimes found it difficult to define the type of cell in the micropore filter. Thus, the response of lymphoid cells in these experiments cannot be ruled out, but the findings suggest that most of the cells have more structural constituents (mitochondria, granules, ribosomal clusters) than do lymphoid cells. The most important question that arises in these experiments, however, is whether or not there are "contaminating" neutrophils participating in the chemotactic response. This possibility appears unlikely for the following reasons: Firstly, the time course of the cell response in these studies is too slow to reflect neutrophil activity. In previous experiments we found that neutrophils complete their response in 90 min (9). In Fig. 1 it is apparent that the cells responding chemotactically are too slow to fit into this pattern. Secondly, the fact that "mononuclear cells" respond to two factors that are not chemotactic for glycogen-induced peritoneal neutrophils (i.e. the factor in treated serum [Fig. 2] and the lysate from neutrophils) indicates that from a functional basis, we are not dealing with the response of neutrophils. Thirdly, the differences in susceptibility to inhibition by phosphonate esters ("cell-dependent inhibition") of mononuclear cells (Fig. 5) as compared to neutrophils (9) indicate that the leukocyte is susceptible to inhibition by phosphonates, but in a manner different from that of neutrophils. Finally, electron microscopic studies prove that we are dealing with a mononuclear cell and not a neutrophil. Thus, it can be firmly concluded that the different chemotactic responsiveness for leukocytes noted in this study reflects the functional behavior of a leukocyte *other* than a neutrophil.

TABLE V

Summary of Chemotactic Factors and their Specificity

Neutrophils	Mononuclear cells
C′(5, 6, 7)a	Factor in serum treated with immune complexes, not C′(5, 6, 7)a
Plasmin-split C′3 fragment	Plasmin-treated fresh serum
Soluble bacterial factors	Soluble bacterial factors
	Lysates from neutrophils

Table V summarizes the current knowledge concerning factors that are chemotactic for neutrophils and mononuclear cells. All of the data were acquired by using the micropore filter technique to study chemotaxis, and this fact should

be kept in mind when comparing the data obtained by other systems. Both neutrophils and mononuclear cells respond to soluble bacterial factors that are identical, since the factor purified by gel filtration (13) was found to be active for both cell types. The factor in plasmin-treated serum to which mononuclear cells respond is not proved to be the C'3 fragment that is active for neutrophils (15). The chemotactic activity in lysates from neutrophils may consist largely of cationic peptides of low molecular weight, which release histamine from mast cells (25, 26). The fact that the chemotactic activity in neutrophil lysates sediments slowly in the ultracentrifuge (Fig. 4) and the finding of chemotactic activity in highly purified cationic peptides (referred to earlier) are both in keeping with this concept. The question can be raised as to why dialysis of neutrophil lysates has little effect on the chemotactic activity (Table IV, experiment B), but Seegers and Janoff are careful to point out that such highly charged substances as the cationic peptides dialyze very poorly in spite of their low molecular weight (27).

The phosphonate esters used in these experiments were studied only for their capacity to induce cell-dependent inhibition of chemotaxis of mononuclear cells. The mononuclear cell is susceptible to irreversible inhibition of chemotaxis by certain of the phosphonates, but the most effective inhibitors (Fig. 5) are not necessarily the same as those that maximally inhibit chemotaxis of the neutrophil (9). This implies that the susceptible serine esterase in the mononuclear cell is not the same as that ("activated esterase") in the neutrophil (19). Whether the mononuclear cell contains a precursor enzyme analogous to the proesterase ("activatable esterase") in the neutrophil (9, 22) that is necessary for the chemotactic response of the leukocyte is not known. This important question awaits further study.

SUMMARY

Chemotaxis of rabbit mononuclear cells was studied by the micropore-filter technique. Mononuclear cells obtained from mineral oil-induced peritoneal exudates respond chemotactically to rabbit serum treated with immune complexes or with streptokinase and plasminogen, to soluble factors produced by bacteria, and to lysates obtained from rabbit neutrophils.

The first chemotactic factor requires heat-labile factors in serum for its generation, but, once formed, the chemotactic factor is relatively heat stable. This factor has been compared with the complement-associated factor in serum, C'(5, 6, 7)a, that is chemotactic for neutrophils. The ability to generate the mononuclear cell chemotactic factor in serum that has been treated with potassium thiocyanate suggests that complement is not required. The position of the chemotactic factor in preparative electrophoresis and density-gradient ultracentrifugation indicates that on the basis of physical-chemical criteria, this factor is not C'(5, 6, 7)a.

The mononuclear cell chemotactic factor present in lysates of neutrophils sediments slowly in the ultracentrifuge and may be related, at least in part, to cationic peptides of lysosomal granules.

A study in the time course of the chemotactic response of mononuclear cells reveals that the response begins to level off after 4 or 5 hr. This is in sharp contrast to the time course for the chemotactic response of neutrophils, in which the reaction is complete within 1.5 hr.

Rabbit mononuclear cells obtained from a starch-induced peritoneal exudate respond to serum treated with plasminogen and streptokinase and to the soluble factor produced by bacteria, but no chemotactic response is elicited to serum treated with immune complexes. This indicates a functional difference between two populations of mononuclear cells. Rabbit alveolar macrophages respond poorly to all agents tested, although a weak chemotactic response to bacterial factors was found.

The requirement of a serine esterase in the mononuclear cell for the cell to respond chemotactically was defined by the use of organophosphorus inhibitors. Pretreatment of mononuclear cells with several series of phosphonates renders them unresponsive in the chemotactic system. The effect is similar for mononuclear cells responding chemotactically to activated serum and to the bacterial chemotactic factor. Several points of contrast with inhibition profiles obtained in the chemotactic system of the neutrophil suggest that, while the mononuclear cell requires a serine esterase for chemotactic responsiveness, this enzyme is different from the one previously defined in the neutrophil.

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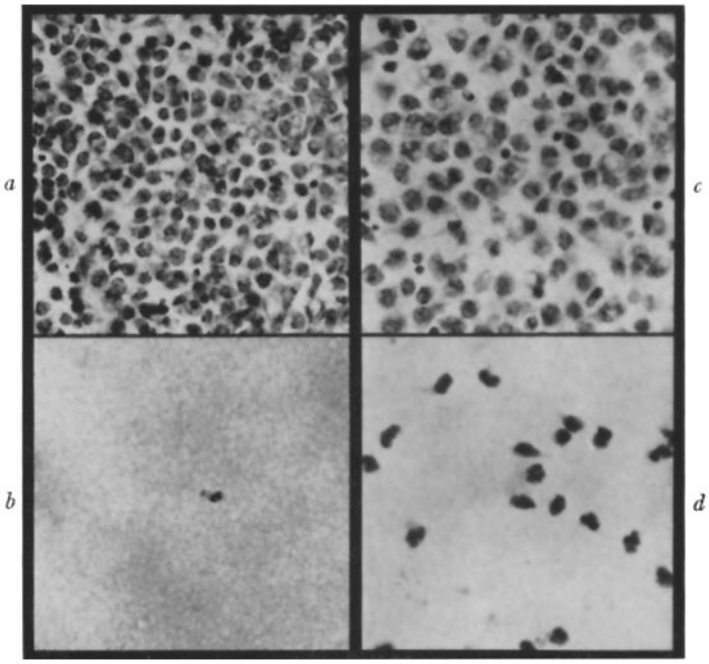

FIG. 6. Photomicrographs of two filters, from a positive and a negative chemotaxis chamber. Frame *a* consists of a field from the upper surface of the filter, where numerous mononuclear cells are present in a monolayer. In frame *b*, the same filter is pictured with focus on the opposite surface of the filter. A single cell has moved through the interstices of the filter. In contrast, while the upper monolayer of nonresponding cells (frame *c*) contains slightly fewer cells, the obvious response is seen by the numerous cells that have moved completely through the filter (frame *d*). Hematoxylin stain. × 400.

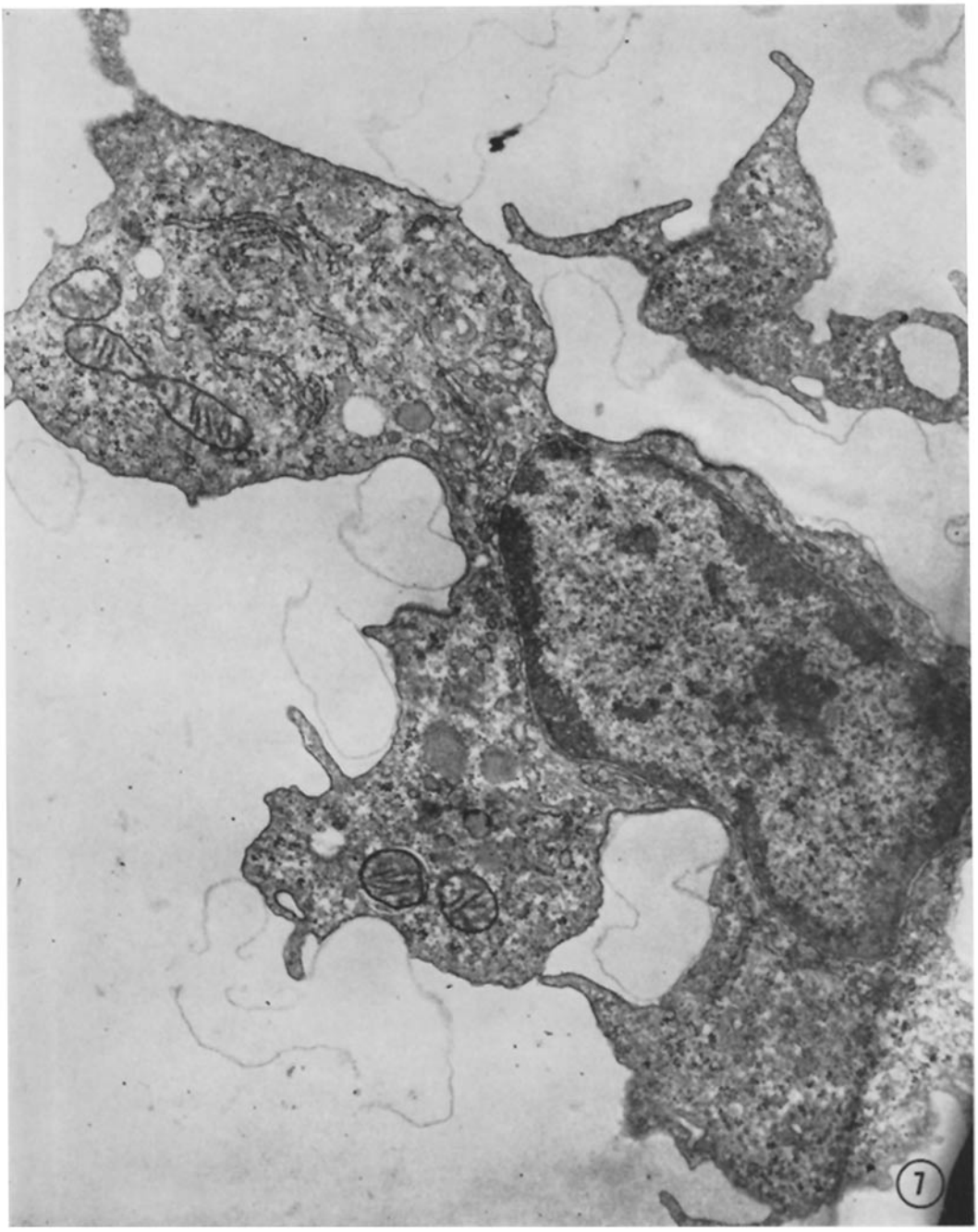

FIG. 7. Electron micrograph of a mononuclear cell within the micropore filter responding to the soluble bacterial chemotactic factor. Typically, these cells become distorted in the process of migrating through the interstices of the filter. Note abundant cytoplasm with mitochondria, endoplasmic reticulum, and electron-opaque bodies. The area outside the cell contains tubular outlines representing solid portions of the filter. Glutaraldehyde fixation. × 16,000.