Migration of Mouse Lymphocytes *in Vitro* from Capillary Tube Cultures[1] (34321)

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During the past 5 years, inhibition of "macrophage" migration in culture medium has been utilized increasingly as an *in vitro* correlate of delayed or cellular hypersensitivity (1–10). The macrophage has generally been considered the exclusive cell type functioning in such migration-inhibition studies (2–4). For example, experiments have been performed in which macrophage-rich preparations were separated from other cell types and used as the indicator for migration-inhibition (2–3). In such studies lymphocyte-rich cell populations were used primarily as the source of "immunologic information" for the specific reaction, but were not considered suitable for migration.

Despite the relatively large number of recent studies concerned with the macrophage as the migrating cell, earlier reports by various investigators had in fact indicated that lymphocytes are indeed motile and that such cells can readily migrate *in vitro*, as well as *in vivo* (11–15). In this regard, Rabinowitz recently reported that purified lymphocyte preparations, separated by glass column adsorption procedures, are a population of highly motile cells as seen *in vitro* in "hanging drops" by means of phase contrast microscopy (16).

Studies in this laboratory with the migration-inhibition procedure have shown that lymphoid cells derived from mouse bone marrow, thymus, or lymph nodes, as well as from the spleen or peritoneal exudate, migrate rapidly from capillary tubes when cultured *in vitro* as control cell suspensions (19). There was, however, no evident correlation between the degree of migration and the percentage of morphologically distinguishable macrophages in the cell population. The present report describes further some of the characteristics of migration of lymphoid cells derived from various mouse tissues. Furthermore, capillary tube migration was effected equally by lymphocyte suspensions purified by removal of glass adherent cells.

Methods and Materials. Animals. Noninbred NIH albino A mice were used for these studies, as described elsewhere (17).

Preparation of lymphoid tissues. Mice were sacrificed by cervical dislocation and the spleen, peripheral and mesenteric lymph nodes, the thymus, and nonlymphoid organs such as the liver, kidney, or lung, were removed aseptically and placed in approximately 5 ml of cold, sterile Hanks' solution in sterile petri plastes (17). Bone marrow cells were obtained from the long limb bones by aspiration and washing with a needle into Hanks' solution. Peritoneal washings were obtained wth 5–10 ml of sterile Hanks' solution. Blood from the retro-orbital venous puncture was used to obtain peripheral blood leukocytes. Solid organs were "teased" with the needle and forceps to obtain a dispersed cell suspension. Cells from each of the sources were passed through several layers of sterile gauze into 15-ml conical centrifuge tubes, washed 3 times by serial centrifugation in the cold with Hanks' solution, and suspended in sufficient sterile medium 199, containing 10% sterile calf serum, to obtain a 10% (v/v) concentration. Viability was estimated with trypan blue dye and cell counts

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were obtained with a hemocytometer. Differential cell counts were made using Wright's stain.

Purification of lymphocytes by glass bead columns. A method essentially similar to that described initially by Rabinowitz was used to fractionate lymphoid cells from the spleen, thymus, and peritoneal exudates (16). Glass columns (1 × 30 cm) were filled with washed silicone-treated micro-glass beads (approx 100 μ diam). Several ml of a washed cell suspension, in tissue culture medium, was placed on the top of the column for 30 min at 37° and then eluted with 20–30 ml of sterile medium containing 10% plasma. Over 98% of these cells were morphologically similar to lymphocytes and over 95% were viable. Polymorphonuclear leukocytes were then eluted with sterile medium containing 0.01 *M* EDTA. Few, if any, lymphocytes were detected in such an eluate. However, many macrophages were present.

Capillary tube migration procedure. Sterile microcapillary tubes, approximately 1.5-mm diameter and 5-cm length, were sealed at one end with melted paraffin wax. The capillaries were individually filled with a test cell suspension, either directly from various tissues or after glass column fractionation. Five to 10 capillaries were placed in a 10 × 75-mm test tube and centrifuged at 500 rpm for 15 min at 4°. Each capillary was then cut with a diamond point at the interface between the packed cells and the medium and two capillaries were placed in individual Sykes-Moore chambers, 18 mm in diameter. The chambers were filled with 1 ml of Hanks' solution, or other medium, and incubated at 37° for 2–18 hr or longer. The degree of migration was estimated visually (0 to 4+) and the area of migration was determined by planimetry. Polaroid photographs were prepared using obliquely transmitted light and 15–20 times magnification, using a Dynazoom B & L stereo microscope.

Results. Rapid migration of cells occurred from the open end of capillary tubes containing cell suspensions prepared from all of the lymphoid tissues (Table I and Fig. 1). The area of migration was generally 10 mm^2 or more within 18–24 hr of incubation. Peripheral blood leukocytes also migrated, but to a lesser degree. In contrast, cell suspensions from liver, lung, or kidneys of the same mice failed to migrate (Table I).

TABLE I. Migration *in Vitro* from Capillary Tubes of Cells Derived from Mouse Lymphoid vs. Nonlymphoid Tissues.

Cell source	Av migration area (mm^2)[a] 24 hr	48 hr
Peritoneal exudate	14.6 ± 2.5	15.8 ± 4.5
Spleen	15.9 ± 3.6	15.3 ± 6.5
Peripheral lymph nodes	11.2 ± 4.6	15.4 ± 2.9
Mesenteric lymph nodes	13.8 ± 6.1	16.1 ± 3.5
Thymus	16.2 ± 3.1	17.1 ± 5.8
Bone marrow	14.7 ± 5.1	14.9 ± 3.1
Peripheral blood "buffy coat"	9.1 ± 3.6	10.2 ± 1.5
Kidney	<2.0	<2.0
Liver	<2.0	<2.0
Lung	<2.0	2.3 ± 0.8

[a] Migration area of at least 4 chambers, each containing 2 capillary tubes with cells pooled from indicated organ of 5 mice; chambers contained medium 199, plus 10% sterile fetal calf serum, and incubated at 37° for indicated length of time.

Cell viability was essential for active migration, none occurring when the cell suspensions were heated at 56° for 1 hr. Similarly, addition of 10^{-3} or 10^{-4} *M* KCN to the suspending medium resulted in rapid death of the cells, as indicated by viability stain, and absence of migration. Furthermore, packed cell suspensions kept in the cold 2 or 3 days, resulted in loss of viability of over 90% of the cells; there was little or no migration on subsequent culture in capillary tubes.

Spleen, peritoneal, and thymus cell suspensions were passed through glass bead adsorption columns to remove phagocytic cells in order to obtain some idea of the nature of the cells involved in migration. Cells which by morphologic criteria were lymphocytes, and which were eluted from glass bead columns similar to cell fractions considered to contain exclusively lymphocytes, readily migrated *in vitro* (Table II). Cell fractions rich in morphologically distinguishable macrophages

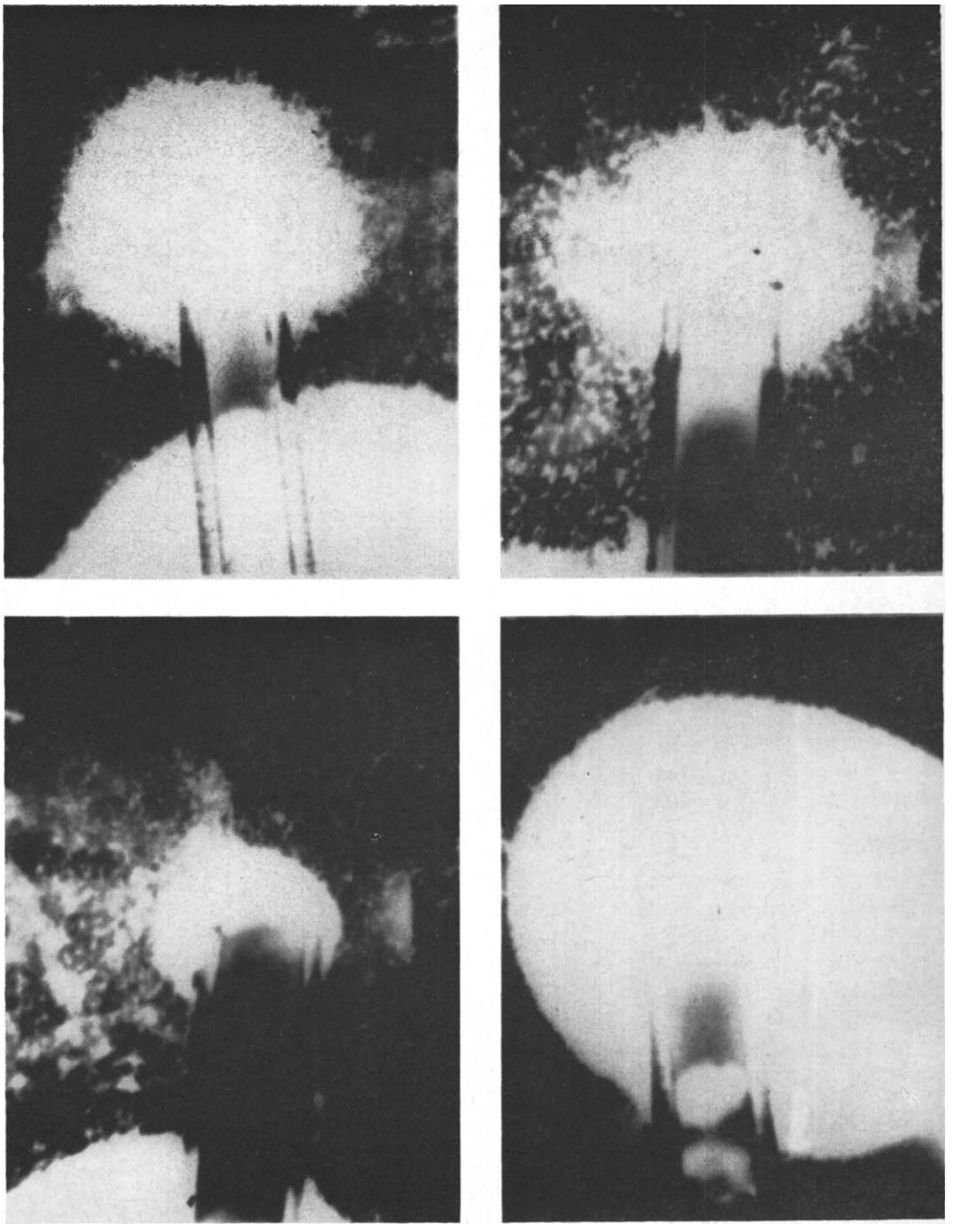

FIG. 1. Lymphoid cell migration from capillary chambers. (upper left), shows migration of mouse spleen cells; (upper right), indicates bone marrow cells; (lower left), shows migration of peripheral "buffy" coat leukocytes; and (lower right), indicates migration of thymus cells. Approximately 5×10^7 nucleated cells placed in each capillary tube and incubated at 37° for 18–24 hr in chambers containing medium 199, plus 10% fetal calf serum.

TABLE II. Column Purification of Spleen, Thymus, and Peritoneal Cell Populations; Effect on Migration of Cells *in Vitro* from Capillary Tubes.

Cell source[a]	Percentage of cell type			Migration area after 24 hr at 37° (in mm²)[b]
	Macrophage-like cells	Lymphocytic cell	Other types	
Spleen				
Nonfractionated	28	59	13	14.3 ± 3.6
Column purified "lymphocytes"	2	87	11	11.2 ± 4.1
Column eluted "macrophages"	86	6	8	13.9 ± 2.6
Thymus				
Nonfractionated	3	93	4	15.8 ± 2.3
Column purified "lymphocytes"	2	98	0	16.3 ± 2.9
Column eluted "macrophages"	Nil	Nil	Nil	—
Peritoneal cells				
Nonfractionated	69	23	8	13.8 ± 5.2
Column purified "lymphocytes"	4	87	9	14.3 ± 3.1
Column purified "macrophages"	92	6	2	16.9 ± 2.2

[a] Cell suspensions, containing approximately 2×10^8 nucleated cells/ml, pooled from 5 or more mice and adsorbed onto micro-glass bead columns, followed by elution with either medium alone, for "lymphocyte-rich" cell population, or with EDTA containing medium for "macrophage-rich" cells.

[b] Average areas of 4 or more chambers, each containing 2 or 3 capillary tube cultures.

likewise migrated. The macrophage-rich cell population readily ingested carbon particles, indicative of phagocytosis, whereas, the lymphocyte-rich cells did so only after a prolonged lag, and more often not at all. In additional experiments, spleen and peritoneal exudate cell suspensions were fractionated by adherence procedures with glass or plastic petri plates, using 3 cycles of adsorption and elution. The lymphocyte-rich population, nonadherent to glass, migrated as well as the "macrophage-rich" fraction which contributed cells adherent to glass surfaces during the three adsorptions. Stains prepared of the migrating cells revealed only a small number of macrophages. Most of the cells in the area of migration were mononuclear, with little cytoplasm, and were not distinguishable from typical lymphocytes in the original cell suspensions.

The compositions of the medium influenced considerably the degree of migration (Table III). When Hanks' salt solution alone, or saline with no addition was used in the chambers, without any supplement, little or no migration occurred. Migration occurred in duplicate chambers containing either Hanks' solution or saline plus 10 or 20% fetal calf serum (Table III). Excellent migration also occurred when complete medium 199, containing calf or mouse serum, was used.

Discussion. Most investigators have considered the "macrophage" to be the principal cell type exclusively involved in the capillary tube migration assay. There seems to be

TABLE III. Effect of Calf Serum on Capillary Tube Migration of Normal Mouse Spleen Cells.

Medium for chambers	Area of migration after 24-hr incubation at 37° (mm²)[a]
Saline alone	<2
Saline + calf serum, 1%	3.8 ± 1.3
10%	4.10 ± 2.6
20%	9.6 ± 2.1
Hanks' solution alone	<2
Hanks' solution + calf serum, 1%	6.8 ± 2.1
10%	14.3 ± 3.2
20%	12.2 ± 2.7

[a] Average results of 4 or more chambers, each containing 2 or 3 capillary tubes.

little question that macrophage-rich suspensions from normal or sensitized individuals can be used as the "indifferent" indicator for *in vitro* delayed hypersenitivity-type reactions. However, the marked motility of other leukocytes has been apparently overlooked or ignored by the many investigators utilizing the capillary tube procedure.

The present work showed that cells derived from various lymphoid tissues rapidly migrate *in vitro* from capillary tubes in tissue culture medium. It remains to be determined whether lymphoid cell suspensions, essentially free of detectable macrophages, can be used as an indicator for delayed hypersensitivity reactions *in vitro*. It should be noted, however, that Carpenter *et al.* (24) recently reported that lymphocytes migrated from spleen explants on agar medium during the first 24 hr of culture. After 5 days the migrating cells were predominantly macrophages. Antigen inhibited migration of both cell types. However, dispersed spleen or lymph node cell suspensions could not be used, since they did not migrate on the agar plates.

The immunological role or function of non-macrophage migrating cells is unknown. Nevertheless, the fact that purified lymphocytes can migrate *in vitro* should be applicable for a variety of biological studies. For example, it was found in preliminary studies that heterologous antilymphocyte serum can prevent migration of thymus, spleen, bone marrow, or peritoneal cells *in vitro* (19). The relatively low concentrations of such sera, which suffice to prevent migration, suggest that the method may be still another means of assaying the biologic properties of ALS. In addition, several antimetabolites, including 6-mercaptopurine and cyclophosphamide, also inhibited lymphocyte migration *in vitro* (19).

It will be of interest to determine if migration inhibitory factor (MIF), which may be induced when lymphocytes from a sensitized individual are incubated *in vitro* with specific antigen, has any effect on migration of normal lymphocytes. Such studies are in progress in this laboratory, as well as a comparison in this regard of purified lymphocytes derived from various species, such as hamster, rabbit, rat, guinea pig, and man. Further studies with this system should provide an additional useful tool for analysis of immunological and physiological properties of lymphoid cells *in vitro*.

Summary. Cell suspensions from mouse lymphoid organs, including the spleen, deep and superficial lymph nodes, bone marrow, and thymus, and from induced peritoneal exudates, were compared as regards their ability to migrate from capillary tubes under conditions of tissue cultures. Cells from all lymphoid tissues migrated rapidly *in vitro*. Purified lymphocyte suspensions prepared by glass bead column or glass plate adsorption procedures containing few, if any, macrophages migrated just as well as unfractionated cell suspensions. The utilization of lymphocytes for migration procedures *in vitro* for various biologic, immunologic, and physiologic studies are discussed.

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1. Carpenter, R. R., J. Immunol. **91**, 803 (1963).

2. Al-Askari, S., David, J. R., Lawrence, H. S., and Thomas, L., Nature **205**, 916 (1965).

3. David, J. R., Lawrence, H. S., and Thomas, S., J. Immunol. **93**, 244 (1964).

4. David, J. R., Proc. Natl. Acad. Sci. U.S. **56**, 12 (1966).

5. Heilman, D. H., Texas Rept. Biol. Med **21**, 136 (1963).

6. Bloom, B. R. and Bennett, B., Science **153**, 80 (1966).

7. Bennett, B. and Bloom, B. R., Transplantation **5**, 4 (1968).

8. Thor, D. E., Science **154**, 1564 (1964).

9. Thor, D. E., Juresis, R. E., Vas, S. R., Miller, E., and Dray, S., Nature **219**, 455 (1968).

10. Juresis, R. E., Thor, D. E., and Dray, S., J. Immunol., **101**, 823 (1968).

11. Bloom, W., *in* "Handbook of Hematology" (H. Downey, ed.) Vol. 2, p. 1471. Harper (Hoeber), New York (1938).

12. Lewis, W. H. and Webster, J. P., J. Exptl. Med. **33**, 261 (1921).

13. DeBruyn, P., Anat. Record. **93**, 295 (1945).

14. Rebuck, J. N. and Crowley, J. H., Ann. N. Y. Acad. Sci. **59**, 757 (1955).

15. Daniels, J. C., Ritzmann, S. E., and Levin, W. C., Texas Rept. Biol. Med. **26,** 5 (1968).

16. Rabinowitz, Y., Blood **23,** 811 (1964).

17. Friedman, H., J. Immunol. **83,** 254 (1962).

18. George, H. and Vaughan, J. H., Proc. Soc. Exptl. Biol. Med. **111,** 514 (1962).

19. Lee, Y., Friedman, H., and Mills, L., RES, J. Reticuloendothelial Soc. **5,** 551 (1968).

20. McFarland, W., Science **163,** 818 (1969). 887 (1965).

21. McFarland, W. and Heilman, D. H., Nature **205,** 887 (1965).

22. McFarland, W. and Moorhead, J. F., J. Exptl. Med. **124,** 851 (1966).

23. Lewis, W. H., Bull. Johns Hopkins Hosp. **49,** 29 (1931).

24. Carpenter, R. R., Barsales, P. B., and Ganchan, R. P., RES, J. Reticuloendothelial Soc. **5,** 472 (1968).

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