TECHNICAL REPORT
RERF TR 16-80
業 績 報 告 書

AN EVALUATION OF CERTAIN FACTORS WHICH INFLUENCE THE IN VITRO MIGRATION OF SUBPOPULATIONS OF HUMAN LYMPHOCYTES

ヒトリンパ球 subpopulation の試験管内
遊走に影響を与える特定因子の評価

JOHN A. PINKSTON, M.D.
STUART C. FINCH, M.D.

放影研
RERF

RADIATION EFFECTS RESEARCH FOUNDATION
財団法人 放 射 線 影 響 研 究 所

A cooperative Japan - United States Research Organization
日 米 共 同 研 究 機 関

ACKNOWLEDGMENT
謝　辞

We wish to acknowledge the excellent technical assistance of Mr. Shozo Iida, and to thank Dr. Charles C. Brown for his assistance with the statistical analysis.

技術面において多大な御援助をいただいた飯田昭三氏と統計解析に御助力いただいた Charles C. Brown 博士に謝意を表する.

RERF TECHNICAL REPORT SERIES
放影研業績報告書集

The RERF Technical Reports provide the official bilingual statements required to meet the needs of Japanese and American staff members, consultants, and advisory groups. The Technical Report Series is in no way intended to supplant regular journal publication.

放影研業績報告書は，日米専門職員，顧問，諮問機関の要求に応えるための日英両語による公式報告記録である．業績報告書は決して通例の誌上発表論文に代わるものではない.

The Radiation Effects Research Foundation (formerly ABCC) was established in April 1975 as a private nonprofit Japanese Foundation, supported equally by the Government of Japan through the Ministry of Health and Welfare, and the Government of the United States through the National Academy of Sciences under contract with the Department of Energy.

放射線影響研究所（元ABCC）は，昭和50年4月1日に公益法人として発足した．その経費は日米両政府の平等分担とし，日本は厚生省の補助金，米国はエネルギー省との契約に基づく米国学士院の補助金とをもって充てる.

放影研 RERF RADIATION EFFECTS RESEARCH FOUNDATION
財団法人 放射線影響研究所

Research Project 研究課題 12-78

AN EVALUATION OF CERTAIN FACTORS WHICH INFLUENCE THE IN VITRO MIGRATION OF SUBPOPULATIONS OF HUMAN LYMPHOCYTES

ヒトリンパ球 subpopulation の試験管内
遊走に影響を与える特定因子の評価

JOHN A. PINKSTON, M.D.[1]; STUART C. FINCH, M.D.[2]

Department of Medicine[1] and Vice-chairman & Chief of Research[2]
臨床部[1], 副理事長・研究担当理事[2]

SUMMARY

The effect on the migration of subpopulations of human lymphocytes of variations in a number of factors known to influence granulocyte migration in the agarose plate system is described. The number of cells and the concentration of serum in the media had a profound effect on the migration of all lymphocyte subpopulations. However, the characteristics of the migration surface and various constituents of the agarose such as the type of serum or the addition of antibiotics or glutamine appeared to have little effect. Factors associated with homologous and heterologous sera which significantly affect granulocyte migration had little influence on the extent of lymphocyte migration, suggesting that there is some difference in motility mechanisms among these cells.

INTRODUCTION

The agarose plate method for the study of granulocyte and monocyte migration in vitro[1-4] has recently been extended to the study of lymphocyte motility.[5] The morphological appearance and migration patterns of T and B cells using this technique have been described.[6,7] In this report, attention is focused on several variables which are of great practical importance in the use of this method for studying lymphocyte motility. Alteration of some variables had a profound effect on the extent of lymphocyte migration. In addition, some factors found to influence granulocyte migration in prior studies failed to appreciably influence lymphocyte migration.

要 約

アガロース平板法において顆粒球の遊走に影響を及ぼすことが知られている多くの因子における変動が，ヒトリンパ球subpopulationの遊走に与える影響について述べた．細胞数及び培養液中の血清濃度は，すべてのリンパ球subpopulationの遊走に大きな影響を与えた．しかしながら，遊走表面の性質，及び血清の種類，抗生物質やグルタミンの添加などアガロースの種々の組成はほとんど影響を及ぼさないようであった．顆粒球の遊走に有意な影響を与える同種血清及び異種血清に関連ある因子は，リンパ球の遊走にはほとんど影響を及ぼさなかった．これは，これらの細胞の運動機序に若干の差異があることを示唆する．

緒 言

顆粒球及び単球の遊走検査に使用されるアガロース平板法[1-4]は，最近，リンパ球遊走検査にも応用されてきた．[5] この技法を用いたT及びB細胞の形態とその遊走像については既に報告した．[6,7] 本報では，リンパ球の運動の検査にアガロース平板法を実際に用いる上で特に重要な数種の変数について検討した．幾つかの変数の変動はリンパ球の遊走範囲に重大な影響を与えた．更に，以前の調査において顆粒球遊走に影響を及ぼすことが判明した若干の因子は，リンパ球遊走に対しては認め得るほどの影響を与えなかった．

Approved承認10 December 1980

Printed 印刷 April 1981

MATERIALS AND METHODS

Materials

Agarose A-45 (agarose) was purchased from Nakarai Chemicals, Ltd., Kyoto, Japan; sheep red blood cells (SRBC) from the Japanese Biological Materials Center, Tokyo, Japan; Medium RPMI-1640 (RPMI) from Nissui Seiyaku Co., Ltd., Tokyo, Japan; and Amphotericin B as Fungizone from ER Squibb and Sons, Inc., Princeton, New Jersey. Pooled human serum (PHS) and human serum albumin (HSA) as Pentax were purchased from Miles Laboratories, Inc., Elkhart, Indiana, and fetal calf serum (FCS) as well as horse serum (HS) from Grand Island Biological Co., Grand Island, New York. Plastic petri dishes 60 × 15 mm were obtained from Falcon, Oxnard, California; Conray 400 (Conray) from Daiichi Seiyaku Co., Tokyo, Japan, and Ficoll-400 (Ficoll) from Pharmacia, Uppsala, Sweden.

Separation of Cells

Ten ml of heparinized venous blood (20 IU heparin/ml) was obtained from each of several healthy adult donors, and mixed with 10 ml of calcium- and magnesium-free balanced salt solution (BSS). Five ml of the diluted blood was layered over 3.0 ml of Ficoll-Conray solution in conical centrifuge tubes. Granulocytes and mononuclear cells were then separated according to the method of Boyum,[8] using sterile technique.

The mononuclear cell suspensions were pooled and washed twice, each time by adding 10.0 ml of BSS followed by centrifugation of 450 G for 10 minutes at 4°C. The cells were suspended in 1.0 ml of BSS and the T lymphocytes were separated using a modification of the method of Greaves and Brown.[9] One ml of a 1% suspension of SRBC was added to the mononuclear cell suspension, which was then divided into 10 aliquots of 0.2 ml each in small test tubes. After standing at room temperature for 15 minutes, the tubes were centrifuged at 20 G for 5 minutes and placed in an ice water bath for 1-2 hours.

The total contents of all the tubes were layered over 3.0 ml of Ficoll-Conray solution and centrifuged at 400 G for 20 minutes at 4°C. The nonrosetting mononuclear cell ring was pipetted off and washed twice, each time with 10.0 ml of BSS followed by centrifugation at 400 G for 10 minutes at 4°C. The T cell-SRBC pellet was lysed with sterile distilled water, and the T cells

材料及び方法

材　料

使用材料及びその購入先は下記のとおりである：アガロースA-45-半井化学薬品，京都；ヒツジ赤血球（SRBC）-日本生物学材料センター，東京；培養液RPMI-1640（RPMI）-日水製薬，東京；Fungizoneとして Amphotericin B-E. R. Squibb and Sons, Inc. New Jersey 州 Princeton 市；Pentaxとしてプールしたヒト血清（PHS）とヒト血清アルブミン（HSA）-Miles Laboratories, Inc., Indiana 州 Elkhart 市；仔ウシ胎児血清（FCS）とウマ血清（HS）-Grand Island Biological Co., New York 州Grand Island 市；プラスチック・ペトリ皿60×15mm-Falcon, California 州 Oxnard 市；Conray 400（Conray）-第一製薬，東京；Ficoll-400（Ficoll）- Pharmacia, Sweden, Uppsala 市.

細胞の分離

数人の健康な成人からヘパリン添加静脈血（ヘパリン20IU/ml）を10ml ずつ採取し，カルシウム及びマグネシウムを含まない平衡化食塩水（BSS）10ml と混合した．希釈した血液 5 ml を円錐形の遠心分離用試験管内で3.0ml の Ficoll-Conray 液上に重層した．次に，顆粒球と単核球を滅菌技法使用の Boyum 法[8] に従って分離した．

単核球浮遊液をプールし，BSS 10.0ml を加えて2回洗浄し，4°C，450Gで10分間遠心分離した．この細胞を1.0ml の BSS に懸濁し，Greaves 及び Brown[9] の変法を用いてTリンパ球を分離した．単核球懸濁液に1%の SRBC 懸濁液 1 ml を加え，小試験管10本に0.2ml ずつ分注した．室温で15分間静置した後，20Gで5分間遠心分離し，氷水槽に1－2時間静置した．

全試験管の全内容液を3.0ml の Ficoll-Conray 液上に重層し，400G，4°Cで20分間遠心分離した．非ロゼッテ単核球細胞環をピペットで取り出し，10.0ml の BSS で2回洗浄した後，400G，4°Cで10分間遠心分離した．T細胞-SRBC ペレットを無菌蒸留水で溶解し，T細胞を上述の非ロゼッテ細胞に用いたと

were then washed in the same manner as described above for the nonrosetting cells.

Monocyte contamination in the nonrosetting cell fraction in some experiments was reduced by glass adsorption to less than 5% as estimated by nonspecific esterase stain.[5,6] The procedure for monocyte adsorption involved resuspension of the nonrosetting cells in 3.0 ml of RPMI in a glass culture flask at 37°C for 30 minutes in a 5% CO_2 incubator. The nonadherent cells in the supernatant were pipetted off, and washed twice in the same manner as described above. The T cells, nonrosetting cells, and monocyte-adsorbed preparations then were suspended in RPMI at various concentrations ranging from 1×10^5 to 2×10^6 cells per 10μl. Viability of cells following washing was greater than 97% by trypan blue exclusion.

Preparation of Agarose Plates

The agarose mixture was prepared by mixing equal amounts of an agarose solution and a nutrient solution. To prepare 10 ml of agarose mixture, 5 ml of agarose solution was prepared by adding 100.0 mg of agarose to 5.0 ml of sterile distilled water, and dissolved by heating.

Approximately 5 cc of nutrient solution was prepared by mixing 0.8 ml RPMI (10× concentrated), 0.1 ml glutamine solution (consisting of 0.3 g glutamine in 10.0 ml sterile BSS), 0.005 ml Amphotericin B solution (consisting of 50.0 mg Amphotericin B in 25.0 ml sterile BSS), 0.025 ml antibiotic solution (consisting of a mixture of 100,000 units of potassium penicillin in 9.0 ml of sterile BSS and 0.2 g streptomycin in 1.0 ml sterile BSS), and 4.2 cc of a serum-sterile distilled water mixture.

The serum concentration in the agarose mixture was varied by changing the serum:sterile distilled water ratio of the nutrient solution, which permitted preparations containing up to 40% serum. For some experiments, the glutamine solution or antibiotic solution was omitted. Either PHS, FCS, HS, or autologous serum was used as the serum component in the nutrient solution. One percent HSA was used instead of serum in some studies. In some experiments, heat inactivation of autologous sera was accomplished by heating the sera at 56°C for 30 minutes prior to incorporation into the nutrient solution.

The heated agarose solution was added to the

同様の方法で洗浄した.

実験によっては，非ロゼッテ細胞分画中に混入した単球は，ガラス吸着を用いて非特異的エステラーゼ染色による推定で5％未満におさえた.[5,6] 単球吸着は，非ロゼッテ細胞をガラス製の培養フラスコを用いて，3.0ml のRPMIで再懸濁し，30分間37℃の5％CO_2恒温器に入れて行った．上澄み中の付着していない細胞をピペットで吸い上げ，上記の方法で2回洗浄した．次に，T細胞，非ロゼッテ細胞及び単球吸着標本を10μl 当たり細胞数1×10^5個から2×10^6個までの色々な濃度でRPMI中に懸濁した．洗浄後の細胞の生育力をトリパン・ブルー排除検査で測定した結果は97％以上であった.

アガロース平板の作成

同量のアガロース液と培養液を混合し，アガロース混合液を作った．10ml のアガロース混合液を作るために，100.0mg のアガロースを5.0ml の無菌蒸留水に加え，加熱して溶解させて5ml のアガロース液を作った.

RPMI（10倍に濃縮）を0.8ml，グルタミン溶液を0.1ml（無菌BSS 10.0ml 中にグルタミンを0.3g添加），Amphotericin B溶液を0.005ml（無菌BSS 25.0ml 中にAmphotericin を50.0mg 添加），抗生物質液を0.025ml（無菌BSS 9.0ml 中にペニシリン・カリウムを10万単位加えたものと無菌BSS 1.0ml 中にストレプトマイシン0.2g を加えたものを混合），及び血清と無菌蒸留水の混合液4.2ccを合わせて約5 ccの培養液を作った.

アガロース混合液の血清濃度は，培養液の血清と無菌蒸留水との比率を変えることによって変化したが，血清濃度40％のものまで作成できた．実験によってはグルタミン溶液若しくは抗生物質液を加えなかったものもある．培養液中の血清成分としてはPHS, FCS, HS若しくは自己血清を用いた．血清の代わりに1％のHSAを用いた検査もあった．実験によっては，自己血清を培養液に加える前に56℃で30分間加熱して，自己血清の熱による非活性化を完全にした.

加熱したアガロース液を培養液に加え，それによって

nutrient solution, and 5 ml of the resulting agarose mixture was poured into a plastic petri dish. In experiments designed to study migration on a glass surface, a sterile 76 × 26 mm glass microscopic slide was placed onto the bottom of a sterile glass petri dish. The warm agarose mixture was then layered over the surface of the glass slide to a thickness of about 2 mm using a sterile Pasteur pipette. The agarose was hardened by refrigeration at 4°C. Circular wells, each 3.0 mm in diameter, and the edges of which were at least 10.0 mm apart, were cut in the hardened semisolid agarose down to the agarose-glass or -plastic interface. The agarose plug was then carefully removed by gentle suction through a Pasteur pipette.

Migration Procedure

A 10μl aliquot, containing from 1×10^5 to 2×10^6 cells suspended in RPMI, was carefully placed into the wells by means of a capillary pipette and the dishes were placed into a humidified 5% CO_2 incubator at 37°C. At the end of 72 hours, the dishes were removed and fixed with Carnoy's solution for approximately 30 minutes. The agar was gently peeled off from the glass or plastic surface with a thin glass coverslip; then, the preparation was washed with tap water and stained with Wright-Giemsa stain.

Quantitation of Migration

Migration distances on stained preparations were measured using a tabletop 35 mm slide projector with a ground glass screen which provided 20-fold magnification. The pattern of lymphocyte migration under agarose gel has been previously described in detail.[5-7] The cells migrate outward from the well radially along the interface of the agarose gel and surface of the slide or petri dish as a monolayer. After 48-72 hours, the cells in most preparations have achieved maximum migration, and the majority of cells appear viable and morphologically intact. For each well, the distance from the edge of the well to the outer zone of migration was measured in four directions, at 90° intervals, and the average was calculated as the distance for that well. For each individual studied, at least two migration wells were used for controls and each variable under study, and the average values were taken as the final endpoint.

Statistical Analysis

Statistical analysis was performed using the paired t-test and regression analysis.

できたアガロース混合液5 ml をプラスチックのペトリ皿に注入した．ガラス面上の遊走を調べるための実験では，76×26㎜の顕微鏡用スライドを殺菌したガラス製のペトリ皿の上に置いた．次に暖めたアガロース混合液をガラスのスライド表面に殺菌したパスツール・ピペットを用いて約2㎜の厚さに重層し，4°Cで冷却してアガロースを固めた．固まった半固体のアガロースに最低10.0㎜間隔で直径3.0㎜の円形試料孔を，アガロースとガラスあるいはプラスチックとの付着面までくりぬき，パスツール・ピペットで静かに吸引して，そのアガロース栓を注意深く取り除いた．

遊走手順

RPMIに1×10^5個から2×10^6個の細胞を浮遊した液10μl を毛細管ピペットで注意深く試料孔に注入し，ペトリ皿を高湿の5％CO_2恒温器中に37℃で静置した．72時間後にペトリ皿を取り出し，Carnoy液中に約30分間固定した．薄いカバーグラスで寒天をガラス若しくはプラスチック面から静かにはがし，標本を水道水で洗浄し，Wright-Giemsa 染料で染色した．

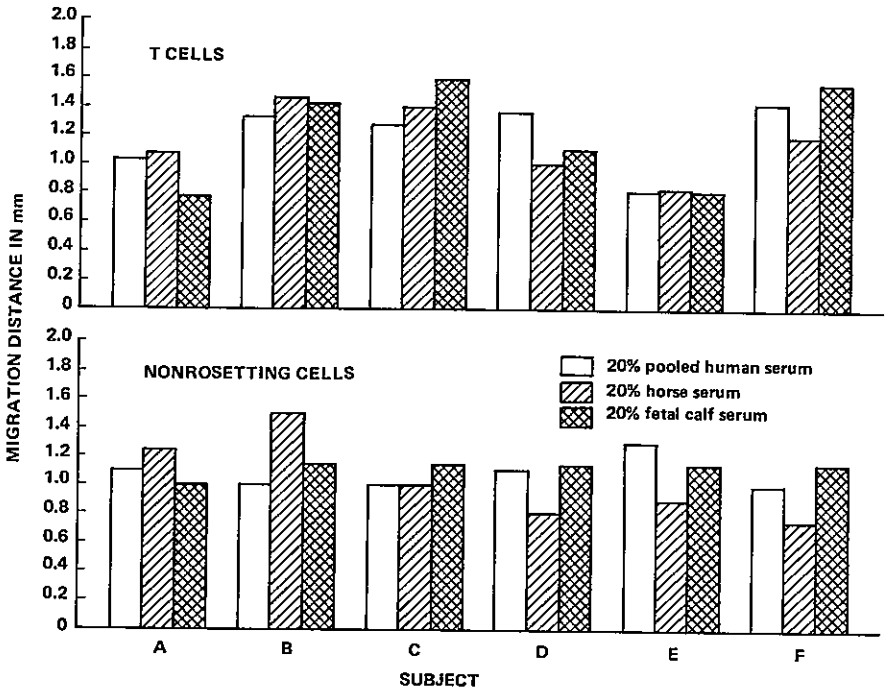
遊走の測定

染色した標本の遊走距離は，すりガラス製スクリーン付の卓上型35㎜スライドプロジェクターを用いて20倍の倍率で測定した．アガロースゲル下のリンパ球遊走像については先に詳述した．[5-7] 細胞はアガロースゲルとスライド若しくはペトリ皿との界面に沿って単一層で試料孔から放射状に遊走する．48—72時間後にはほとんどの標本で細胞が最大の遊走を示し，その大部分が生存しており，形態は損なわれていないようである．各試料孔の端から最大遊走範囲までの距離を90度間隔で4方向で測定し，その平均値を遊走距離とした．対象者1人に関して，対照及び各変数について少なくとも2個の試料孔を用い，平均値を最終成績値とした．

統計学的解析

統計学的解析は対 t 検定と回帰解析を行った．

FIGURE 1 INFLUENCE OF SERUM TYPE IN MEDIA ON MIGRATION OF HUMAN MONONUCLEAR CELLS, 72 HOURS OF INCUBATION
図1　培養液中の血清の種類がヒト単核球の遊走に及ぼす影響，72時間培養



RESULTS

The results of using various sera as well as HSA incorporated into the agarose medium are shown in Figure 1. Twenty percent PHS, FCS, and HS, as well as 1% HSA, were compared. Each well contained 1×10^6 cells, and studies were completed on six individuals. No significant difference in migration distance for either the T or nonrosetting cells was observed based on type of serum used; for the nonrosetting cells, however, when PHS and FCS were compared, the results were of borderline significance ($.05<p<.10$). No migration occurred in any of the preparations when HSA was substituted for serum.

The results of varying the serum concentration in the agarose medium are shown in Figure 2. PHS was used, with 1×10^6 cells per well, and studies were completed on four individuals. The serum concentrations tested ranged from 5%-40%. The achieved migration distance increased as a function of increasing serum concentration over the range tested. The results were highly significant for both T cells ($p<.001$) and non-

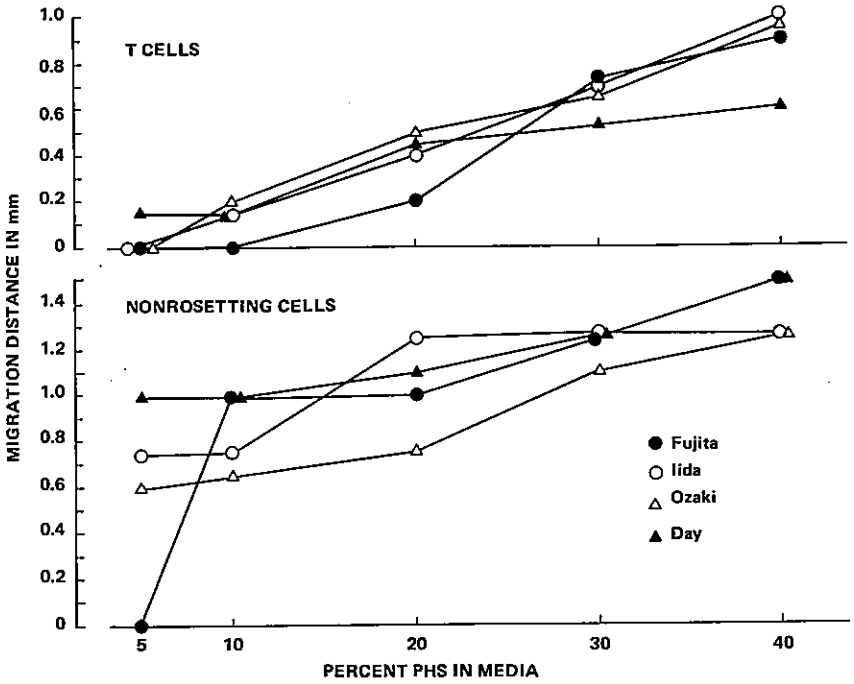
結　果

アガロース培地にHSAや種々の血清を加えた結果を図1に示した．1％HSAや20％のPHS，FCS及びHSを用いた場合の結果を比較した．各試料孔には1×10^6個の細胞を入れ，6例について調査を行った．T細胞や非ロゼッテ細胞の遊走距離に使用した血清の種類による有意差は見られなかった．しかし，非ロゼッテ細胞については，PHSとFCSを比較すると境界線的有意の差異が見られた（$.05<p<.10$）．血清の代わりにHSAを用いた場合は，どの標本も遊走が見られなかった．

アガロース培地の血清濃度を変化させた場合の結果を図2に示した．PHSは各試料孔に細胞1×10^6個を入れて使用し，4例について調査を行った．血清濃度は5％—40％であった．この範囲では，遊走距離は血清濃度の関数として増加した．T細胞（$p<.001$）及び非ロゼッテ細胞（$p<.001$）の双方とも高い有意

FIGURE 2 HUMAN MONONUCLEAR CELL MIGRATION AT 72 HOURS IN FOUR SUBJECTS AS A FUNCTION OF PERCENT PHS IN MEDIA

図2　培養液中の PHS の割合(%)とヒト単核球遊走との関数関係，4人の対象者，72時間培養



rosetting cells ($p<.001$). Nonrosetting cell migration exceeded T cell migration at each concentration tested.

The value of adding glutamine to the agarose mixture was studied. The migration distances for T and nonrosetting cells, with and without additional glutamine, were compared for six subjects. For all studies there was 20% PHS in the agarose, and 1×10^6 cells were placed in each well. The average migration distances were nearly identical for each subject (results not shown).

The influence of both heat labile and heat stable autologous serum factors, as well as antibiotics, on the extent of T lymphocyte migration was studied in four subjects, and the results are shown in Figure 3. Heat inactivated autologous serum with and without antibiotics, autologous serum with antibiotics, and PHS with and without antibiotics were compared. The serum concentration was 20% and the number of cells per well was 1×10^6. No significant difference was noted for any of these variables, indicating that results

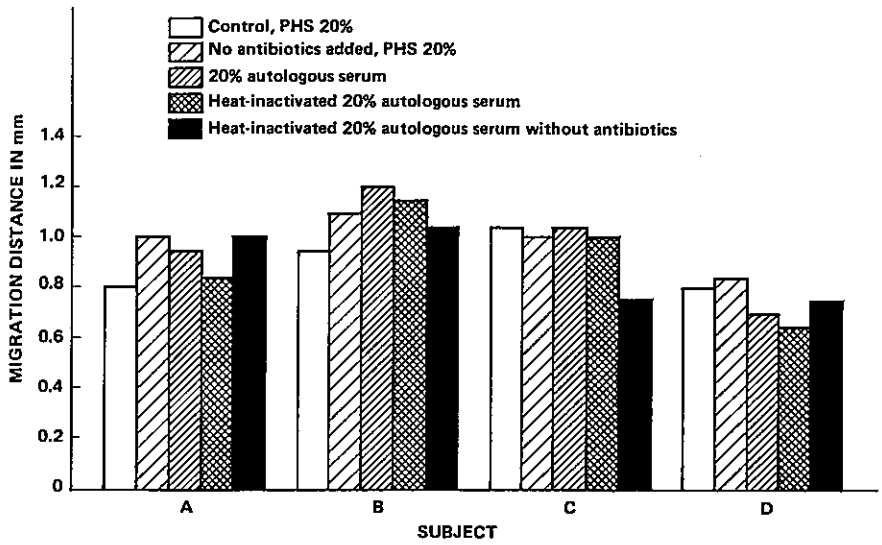
性を示した．各血清濃度において，非ロゼッテ細胞の遊走はT細胞よりも大きかった．

アガロース混合液にグルタミンを添加した場合の影響を調べた．6例について，グルタミンを添加した場合としない場合のT細胞及び非ロゼッテ細胞の遊走距離を比較した．実験はすべて，アガロースに20%の PHS を加え，各試料孔に 1×10^6 個の細胞を入れて行った．平均遊走距離は各対象者ともほぼ同じであった(結果は表示していない)．

抗生物質だけでなく，熱不安定自己血清因子と熱安定自己血清因子がTリンパ球の遊走範囲に与える影響について4例を用いて調べた．その結果は図3に示した．熱非活性化自己血清に抗生物質を加えた場合と加えない場合，抗性物質を加えた自己血清，及び PHS に抗生物質を加えた場合と加えない場合を比較した．血清濃度は20%，各試料孔当たりの細胞数は 1×10^6 個とした．これらの変数については有意差は認められず，熱による非活性化処理を

FIGURE 3 EFFECT OF AUTOLOGOUS SERUM, ANTIBIOTICS, & HEAT-INACTIVATED SERA ON THE MIGRATION OF HUMAN T CELLS, 72 HOURS OF INCUBATION

図3　自己血清，抗生物質及び熱非活性化血清が
ヒトT細胞の遊走に及ぼす影響，72時間培養



using autologous serum with or without heat inactivation are comparable to PHS, and further, that the results are not significantly influenced by antibiotics.

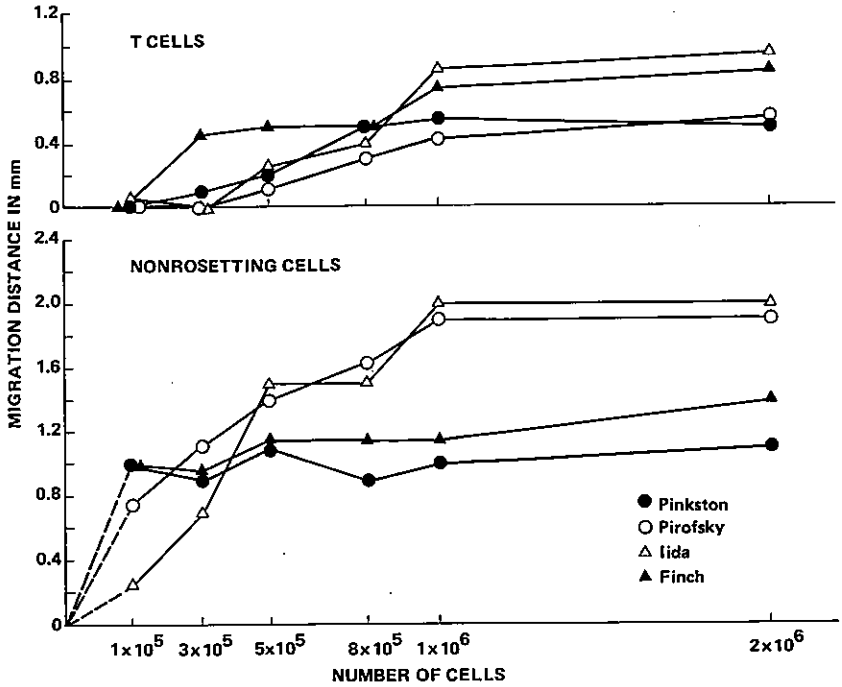
The effect on the migration distance of varying the number of cells in the well was evaluated for four subjects (Figure 4). Cell number was varied from 1×10^5 to 2×10^6 in a constant volume of $10\mu l$ of suspending medium. Variation in the number of cells placed into the wells significantly influenced the results obtained. The increase in migration observed with an increase in cell number from 1×10^5 to 1×10^6 was significant for both T cells ($p<.01$) and nonrosetting cells ($p<.01$). No further increase was observed, however, for the cells in either preparation, when the number of cells per well was increased from 1×10^6 to 2×10^6. T cell migration was erratic when fewer than 5×10^5 cells were used, with virtually no migration occurring with less than 3×10^5 cells. In contrast, the nonrosetting cells migrated further than the T cells at the lower cell concentrations. Detectable nonrosetting cell migration consistently occurred with 1×10^5 cells.

行った自己血清と行わない自己血清を用いた場合の結果はPHSと匹敵し，更に実験結果は抗生物質によって有意な影響を受けないことを示した．

試料孔内の細胞数の変化が遊走距離に与える影響を4例について評価した（図4）．浮遊培養液の量を一定（$10\mu l$）にし，細胞数を 1×10^5 個から 2×10^6 個まで変化させた．試料孔内の細胞数の変化は，得られる結果に有意な影響を与えた．細胞数を 1×10^5 個から 1×10^6 個に増やした場合の遊走の有意な増加はT細胞（$p<.01$）及び非ロゼッテ細胞（$p<.01$）の双方で見られた．しかし，試料孔当たりの細胞数を 1×10^6 個から 2×10^6 個まで増やしても，どの標本もそれ以上の遊走の増加はなかった．T細胞の遊走は，細胞数が 5×10^5 個未満の場合は不規則であり，3×10^5 個未満では事実上遊走は起こらなかった．これに反して，非ロゼッテ細胞は，少ない細胞数でもT細胞より遊走が活発であった．非ロゼッテ細胞の探知可能な遊走は一貫して 1×10^5 個で見られた．

FIGURE 4 EFFECT OF CELL NUMBERS ON EXTENT OF HUMAN MONONUCLEAR CELL MIGRATION IN FOUR SUBJECTS, 20% PHS IN AGAR, 72 HOURS OF INCUBATION

図4　細胞数がヒト単核球遊走範囲に及ぼす影響，4人の対象者，培養基中に20% PHS を加えた場合，72時間培養



To compare migration on glass and plastic surfaces, preparations on both glass microscope slides and plastic petri dishes were made simultaneously from six individuals. T cell, nonrosetting cell, and monocyte adsorbed nonrosetting cell populations were studied (Table 1). No significant difference in migration distance by type of surface was demonstrated for any of the three cell populations studied. Although the T cells from most subjects migrated further on glass than plastic, the results of paired t-testing were not statistically significant (p>.10).

Variation in T cell and nonrosetting cell migration distances was determined for seven persons (Table 2). For each individual in the study, both T and nonrosetting cells were tested at different times under the same experimental conditions, using the basic method previously described.[5] The time between the individual experiments (A-E) varied from several days to several weeks. Experiments A-E were done sequentially so that A, B, and C preceded D and E. Some variation was seen in the same subject as well as groups consisting of the same subjects

ガラス面とプラスチック面における遊走を比較するために，ガラス製の顕微鏡用スライドとプラスチック製のペトリ皿の双方で同時に6人の標本を作った．T細胞，非ロゼッテ細胞，単球吸着非ロゼッテ細胞の各 population について調べた（表1）．この三つの population のいずれにおいても遊走表面の種類によって遊走距離に有意な差異は見られなかった．ほとんどの対象者において，T細胞の遊走はプラスチックよりもガラスの方が活発であったが，対 t 検定の結果は統計学的に有意ではなかった（p>.10）．

7人の対象者について，T細胞及び非ロゼッテ細胞の遊走距離の変化を測定した（表2）．各対象者について，前述した基本的技法[5]を用いて，同一の実験条件のもとで時を変えてT細胞と非ロゼッテ細胞の遊走を調べた．それぞれの実験（A-E）は数日から数週間の間隔で行われた．実験Aから実験Eまで，A，B，C，D，Eの順で行った．実験時が異なると同じ対象者を含む群だけでなく同一の対象者に

TABLE 1 HUMAN MONONUCLEAR CELL MIGRATION (mm) ON GLASS AND PLASTIC SURFACES, 72 HOURS OF INCUBATION
表1　ガラス及びプラスチック面上のヒト単核球の遊走(mm)，72時間培養

Subject	T Cells		Nonrosetting Cells		Monocyte Adsorbed Nonrosetting Cells	
	Glass	Plastic	Glass	Plastic	Glass	Plastic
1	0.8	1.0	1.5	1.5	-	-
2	1.0	0.7	2.0	2.0	1.2	1.0
3	1.2	0.9	1.5	1.0	1.4	1.5
4	1.4	1.4	2.0	3.0	1.8	1.5
5	1.3	1.0	2.5	2.0	1.8	1.9
6	1.2	0.5	1.5	1.2	-	-

T cells, $p>.1$; nonrosetting cells, $p>.8$; monocyte adsorbed nonrosetting cells, $p>.4$
T細胞，$p>.1$；非ロゼッテ細胞，$p>.8$；単球吸着非ロゼッテ細胞，$p>.4$

TABLE 2 HUMAN MONONUCLEAR CELL MIGRATION DISTANCE (mm) BY SUBJECT AND EXPERIMENT, 72 HOURS OF INCUBATION
表2　ヒト単核球遊走距離(mm)，対象者及び実験別，72時間培養

Experiment	Subject						
	1	2	3	4	5	6	7
				T Cells			
A	1.0	-	1.3	1.4	1.4	-	0.8
B	-	-	0.8	1.0	1.1	-	-
C	1.1	-	1.3	1.2	1.1	-	-
D	0.6	0.4	0.8	0.7	-	-	-
E	-	-	0.4	-	0.5	0.2	0.5
				Nonrosetting Mononuclear Cells			
A	1.1	-	1.0	1.0	1.1	-	1.3
B	-	-	-	-	-	-	-
C	1.5	-	1.2	2.0	1.5	-	-
D	1.0	1.9	2.0	1.1	-	-	-
E	-	-	1.3	-	1.1	1.0	0.8

when tested at different times. In experiments D and E, the T cell migration distances were generally shorter than in experiments A-C, even though the lymphocytes in many cases were obtained from the same subjects. The mean T cell migration for experiments D and E was 0.51 ± 0.19 mm, and for A-C 1.13 ± 0.21 mm. The difference is statistically significant ($p<.001$). For nonrosetting cells, the mean of experiments D and E (1.28 ± 0.44 mm) is not significantly different ($.8<p<.9$) from the mean of A-C (1.30 ± 0.32 mm).

おいても変化が見られた．実験D及びEにおいては，多くの場合同一対象者から採取したリンパ球であったにもかかわらず，T細胞の遊走距離は概して実験A-Cよりも短かかった．実験D及びEにおけるT細胞の平均遊走距離は0.51 ± 0.19mm，実験A-Cの平均値は1.13 ± 0.21mmであり，その差異は統計学的に有意であった($p<.001$)．非ロゼッテ細胞の遊走は，実験D及びEの平均値(1.28 ± 0.44mm)と実験A-Cの平均値(1.30 ± 0.32mm)との間に有意差はなかった($.8<p<.9$)．

DISCUSSION

The results of these preliminary experiments indicate that the extent of mononuclear cell migration under agarose is profoundly influenced by the serum concentration in the supporting medium and the number of cells placed in the wells. T cells were more influenced by both of these factors than were the nonrosetting cells. T cells showed an almost linear response of increasing maximum migration distance achieved as the serum concentration was increased over the range of 5%-40%, with relatively little migration occurring in the 5%-10% range. In contrast, the nonrosetting cells migrated much better in the presence of serum concentrations in the range of 5%-10% than T cells, but the degree of increased migration thereafter as a function of increasing serum concentration was similar to that observed for T cells. None of the cells migrated in the absence of serum, or when 1% HSA was substituted for serum. The percentage of serum used in the agarose media in these experiments was varied by substituting PHS for distilled water in the nutrient solution which allows the concentration to be varied from 0%-40%. In view of the enhanced migration observed when serum concentration is increased, experiments in which alteration of the medium preparation procedure will permit serum concentrations higher than 40% are planned for the future.

Variation in the number of cells placed in the wells affected the extent of migration for both types of cells. Within a limited range the extent of T cell migration varied directly with the number of cells in the well. Below 5×10^5 cells, however, little or no migration occurred and above 1×10^6 cells no additional migration was observed. Like T cells, nonrosetting cells did not increase migration above a finite number of cells in the well. In contrast to T cells however, nonrosetting cell migration occurred in the presence of relatively few cells in the well ($< 1 \times 10^5$).

Variation of several factors appeared to have little effect on lymphocyte migration. The addition of glutamine did not enhance migration, indicating that levels already present in RPMI medium and serum are sufficient. This finding also implied that increased glutamine was not the factor producing the increased migration found with increasing serum concentration. The type

考　察

これらの予備実験の結果は，アガロース下の単核球細胞の遊走範囲が，支持体中の血清濃度及び試料孔当たりの細胞数によって大きく影響されることを示している．T細胞は非ロゼッテ細胞よりも大きくこの二つの因子に影響された．T細胞は，血清濃度が5％—40％に増加するに伴って最大遊走距離が増加するというほぼ線形の反応を示し，5％—10％ではほとんど遊走が見られなかった．一方，非ロゼッテ細胞は，血清濃度5％—10％においてT細胞よりはるかに活発な遊走を示した．しかし，それ以上の濃度においては，血清濃度の関数としての遊走増加率はT細胞の場合と同じであった．血清を加えなかった場合や血清の代わりに1％のHSAを用いた場合には遊走は見られなかった．これらの実験では，アガロース培地内の血清の割合を培養液中のPHSを蒸留水に代えることによって0％—40％まで変化させた．血清濃度が増加すると遊走が活発になることから，培養液の作成法を変えることによって血清濃度を40％以上にできる実験を将来行うことを計画している．

試料孔内の細胞数の変化がT細胞及び非ロゼッテ細胞の両タイプともその遊走範囲に影響を及ぼした．限られた範囲内では，T細胞の遊走範囲は試料孔中の細胞数と比例して変化した．しかし，細胞数が5×10^5個以下の場合は遊走はほとんどあるいは全く起こらず，1×10^6個以上では，遊走の増加は見られなかった．T細胞と同様に，非ロゼッテ細胞も試料孔中の細胞数が特定の数以上になると遊走の増加は見られなかった．しかし，非ロゼッテ細胞はT細胞と異なり，比較的少ない細胞数（$< 1 \times 10^5$個）で遊走が起こった．

数種の因子における変化は，リンパ球遊走にほとんど影響を与えないようであった．グルタミンの添加は遊走を活発化しなかった．これはRPMI培養液及び血清に既に存在するレベルが十分なものであることを示している．この所見はまた，グルタミンの増加は，血清濃度の増加によって見られるような遊走の増加を引き起こす因子ではなかったことも意味している．被検血清の種類による一定の影響は見られず，

of serum used produced no consistent effect, implying that there are no species-specific chemokinetic or migration inhibition factors among the autologous serum, PHS, HS, or FCS used in these studies. The studies with heat-inactivated serum provided evidence that heat-labile factors do not affect the extent of T lymphocyte migration.

これらの実験で用いた自己血清，PHS，HS若しくはFCSには，種類による化学反応速度や遊走を抑制する因子がないことを意味している．熱による非活性化血清を用いた実験からは，熱不安定因子はTリンパ球の遊走範囲に影響を及ぼさないという証拠が得られた．

Preparations from which antibiotics and Amphotericin B were omitted showed no differences in the extent of T cell migration when compared with controls. Migration on glass or plastic surfaces was comparable for T cells, nonrosetting cells, and the monocyte-adsorbed preparations, so that either glass or plastic surfaces may be used for migration studies by this technique.

抗生物質及びAmphotericin Bを除いた標本は，対照と比較してT細胞の遊走範囲に差異が見られなかった．T細胞，非ロゼッテ細胞及び単球吸着標本は，ガラス面とプラスチック面での遊走が類似していたので，この技法による遊走実験にはガラス面とプラスチック面のどちらも使用できる．

The experiments for this study were carried out over a period of several weeks during which time some variation in T and nonrosetting cell migration was observed in the same individuals tested at different times. Variation in the average mononuclear cell migration distance for groups of individuals tested at different times also was observed. These types of variations probably result from both normal individual variation and technical factors which are as yet poorly understood, and emphasize the need for incorporating controls as an essential part of each experiment.

本調査においては数週間にわたって実験を行ったが，その間，異なる時期に検査を行った同一の対象者のT細胞及び非ロゼッテ細胞の遊走に若干の変化が見られた．異なる時期に検査した群の単核球の平均遊走距離にも変化が見られた．こうした変化は，正常な個体差と技術要因の双方によるものと考えられるが，まだよく解明されていないので，各実験において対照を重要な要素として用いる必要性が強調された．

Several differences in the random migration characteristics of granulocytes and lymphocytes in the agarose system are evident from these and prior studies. Granulocytes rapidly migrate outward within an hour or two of being placed in the well, whereas several hours or days are required for comparable lymphocyte migration to occur.[1-5,7]

今回並びに前回の実験から，顆粒球及びリンパ球の不規則遊走の特性に若干の差異が見られることは明らかである．顆粒球は試料孔に注入してから1－2時間以内に速い速度で外側に遊走するが，リンパ球遊走が起こるには数時間若しくは数日かかった．[1-5,7]

Factors associated with heterologous sera, when compared to homologous sera, did not appear to significantly affect the extent of lymphocyte migration. The chemokinetic activity of granulocytes, however, is profoundly influenced by heterologous serum factors.[2,10] Granulocytes show markedly enhanced chemokinesis in the presence of either fresh or heat inactivated autologous serum or PHS when compared with HS, which in turn, is significantly more chemokinetic than FCS. Granulocytes are similar to lymphocytes, however, in that no significant alteration in chemokinetic activity is

異種血清に関連する因子は，同種血清と比較してリンパ球の遊走範囲に有意な影響を与えなかったようである．しかし，顆粒球の化学反応速度は異種血清因子によって大きく影響を受ける．[2,10] 顆粒球は新鮮な自己血清若しくは熱非活性化自己血清を用いると，HSを用いた時と比較して化学反応速度が顕著に増加するが，HSはFCSより化学反応速度が有意に高い．しかし，新鮮な自己血清と熱非活性化自己血清のいずれを用いても化学反応速度に有意な

found in the presence of either fresh or heat inactivated autologous serum.

Granulocytes, in contrast to lymphocytes, migrate under agarose when 1% HSA is substituted for serum in the media.[1,3,10] In addition, previous studies have shown that granulocyte migration is increased when the medium serum concentration is increased from 0%-10%, but that no further increase occurs with higher concentrations.[10] In contrast, lymphocytes appear to be markedly sensitive to serum concentrations in the range of 5%-40%, with T cells more significantly affected than nonrosetting cells at concentrations in the 5%-10% range.

The extent of both granulocyte and lymphocyte random migration is profoundly influenced by the number of cells placed in the well. For granulocytes, this has been demonstrated not only in the agarose plate system,[1,4,10] but also in filter and capillary tube assay systems.[11] Following instillation into the wells, leukocytes tend to marginate along the side of the well at the agarose surface interface. When smaller numbers of cells are instilled, fewer cells are seen at the margin. Even at low lymphocyte numbers, however, when no migration occurs, there is evidence of cell margination at the agarose surface interface. The reason why migration is impaired in these instances is not well understood. These preliminary observations provide additional evidence that there are basic differences in granulocyte and lymphocyte motility mechanisms in view of the differences in in vitro migration response of these cells to different exogenous factors.[1-7] More widespread application of the agarose plate method for the study of lymphocyte motility may be expected to yield additional information on the underlying motility mechanisms of these cells.

変化がないという点では，顆粒球とリンパ球は同じである．

顆粒球はリンパ球と異なり，培養液中の血清の代わりに１％のHSAを用いてもアガロース下で遊走する．[1,3,10] 更に，以前の調査では培養液中の血清濃度が０％－10％に増加すると顆粒球遊走が増加したが，濃度をそれ以上にしても遊走増加は起こらなかった．[10] 一方，リンパ球は血清濃度５％－40％の範囲では非常に反応性が高く，５％－10％ではT細胞の方が非ロゼッテ細胞よりも有意に影響を受けやすいようである．

顆粒球及びリンパ球双方の不規則遊走範囲は試料孔中の細胞数によって大きく影響を受ける．顆粒球の場合，この現象はアガロース平板法のみならず，[1,4,10] フィルターや毛細管測定法[11] においても認められる．白血球は試料孔に注入後，試料孔側のアガロース界面に沿って遊走する傾向がある．注入する細胞数が少なければ，遊走する細胞数も少ない．しかし，遊走が起こらないような少ない細胞数でもアガロース界面では遊走が起こるという証拠がある．この場合に遊走が阻害される原因はよく分かっていない．更に，これらの予備的観察所見から，顆粒球とリンパ球遊走機序には，異なる外因に対する試験管内の遊走反応に差異がある点からみても，基本的な差異があることが明らかである．[1-7] これらの細胞の基本的な遊走機序に関する更に多くの情報を得るためには，リンパ球遊走検査に広くアガロース平板法を応用することが期待される．

REFERENCES
参考文献

1. NELSON RD, QUIE PG, SIMMONS RL: Chemotaxis under agarose: A new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. J Immunol 115:1650-6, 1975

2. JOHN TJ, SIEBER OF Jr: Chemotactic migration of neutrophils under agarose. Life Sci 18:177-82, 1976

3. REPO H: Leukocyte migration agarose test for the assessment of human neutrophil chemotaxis. I. Effects of environmental factors on neutrophil migration under agarose. Scand J Immunol 6:203-9, 1977

4. REPO H, KOSUNEN TU: Leukocyte migration agarose test for the assessment of human neutrophil chemotaxis. II. Variables in the attraction assay. Scand J Immunol 6:211-8, 1977

5. PINKSTON JA, FINCH SC, IIDA S, CAPLAN RA: An agarose plate method for the study of human T and B lymphocyte migration. Jpn J Exp Med 48:279-82, 1978 (RERF TR 1-78)

6. PINKSTON JA, FINCH SC: A method for the differentiation of T and B lymphocytes and monocytes migrating under agarose. Stain Technol 54:233-9, 1979 (RERF TR 1-79)

7. PINKSTON JA, FINCH SC: Human T and B lymphocyte migration under agarose: Differences in the characteristics of migrating cells and migration patterns. RERF TR 3-79

8. BOYUM A: Separation of blood leukocytes, granulocytes and lymphocytes. Tissue Antigens 4:269-74, 1974

9. GREAVES MF, BROWN G: Purification of human T and B lymphocytes. J Immunol 112:420-3, 1974

10. PINKSTON, JA, FINCH SC: Unpublished observations

11. PATTEN E, GALLIN JI, CLARK RA, KIMBALL HR: Effects of cell concentration and various anticoagulants on neutrophil migration. Blood 41:711-9, 1973