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EFFECT OF COLCHICINE ON HUMAN GRANULOCYTE RANDOM MIGRATION AND CHEMOTAXIS UNDER AGAROSE

ヒト顆粒球のアガロース培地下不規則遊走及び
走化性に及ぼすコルヒチンの影響

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ヒト顆粒球のアガロース培地下不規則遊走及び走化性に及ぼすコルヒチンの影響

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SUMMARY

The effect of various concentrations of colchicine on the migration of human granulocytes over a period of 16 hours was studied by means of a modified agarose plate system. Control random migration and chemotaxis were rapid up to 4-6 hours with little further advance after 8 hours. Chemotactic migration at two hours was inhibited by both 10^{-7}M and 10^{-5}M colchicine, but thereafter, no inhibition was observed. Random migration at a concentration of 10^{-7}M colchicine was also inhibited only at two hours. Significant inhibition of random migration was observed with 10^{-5}M colchicine, however, for migration periods up to six hours.

The early inhibition of chemotaxis and stimulated random migration in the presence of physiologic concentrations of colchicine is consistent with previous evidence that these processes are microtubular dependent. The transient nature of this inhibition, however, suggests that the cells which are slowed continue to migrate longer than the control cells so that the eventual maximum migration distance for the untreated and colchicine-treated cells is about the same.

INTRODUCTION

Colchicine has been shown to inhibit the chemotaxis of granulocytes in vitro,[1-5] but to have little effect on their random motility.[4,5] This inhibition is generally attributed to its antitubulin properties, which cause impairment in the assembly of microtubules, structures of

要 約

アガロースプレート法の変法を使用して，種々の濃度のコルヒチンがヒト顆粒球の遊走に及ぼす影響を16時間培養を行って検討した．対照用の顆粒球の不規則遊走と走化性は，遊走開始後4時間から6時間までは急速であったが，8時間以降はほとんど見られなかった．コルヒチン濃度10^{-7}M及び10^{-5}Mにおいて走化性遊走は2時間の時点で抑制されたが，それ以後抑制は認められなかった．不規則遊走も濃度10^{-7}Mのコルヒチンで2時間の時点でのみ抑制された．しかし，コルヒチン濃度10^{-5}Mにおいては，不規則遊走に有意な抑制が6時間まで認められた．

生理的濃度のコルヒチンの存在下で，走化性と刺激された不規則遊走が早期に抑制されたことは，これらの過程が微小管に依存するという以前報告された所見に一致する．しかしこの抑制が一過性であるため，遊走速度が減じた細胞が対照細胞より長時間遊走を続け，それによってコルヒチン未処理のいかんにかかわらず細胞の最大遊走距離は結果的にほぼ同じであることを示唆する．

緒 言

コルヒチンは顆粒球の試験管内走化性を抑制するが，[1-5] その不規則遊走にはほとんど影響を与えないとされている．[4,5] この抑制は一般に抗小管特性によるものであり，これが走化性運動に重要な役割を

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importance in chemotactic locomotion.[4-6] Most prior studies of the effects of colchicine on granulocyte migration have utilized the Boyden chamber technique or one of its modifications, and incubation periods of 4-5 hours or less.[1,3-5]

The purpose of the present study was to investigate the effect of various concentrations of colchicine on the motility of human granulocytes at various time intervals during migration periods up to 16 hours. It was possible to evaluate both random migration and chemotaxis by means of a modified agarose plate technique.[7-9]

Materials and Methods

Agarose A-45 (agarose) was obtained from Nakarai Chemicals, Ltd., Kyoto, Japan; Medium TC-199 (TC199) from Nissui Seiyaku, Co., Ltd., Tokyo, Japan; fetal calf serum (FCS) from Grand Island Biological Co., Grand Island, New York; colchicine from Wako Pure Chemical Industries, Ltd., Osaka, Japan; and Dextran T500 (Dextran) from Pharmacia, Uppsala, Sweden. Plastic petri dishes 60 × 15mm, were purchased from Falcon, Oxnard, California; Ficoll 400 (Ficoll) from Pharmacia, Uppsala, Sweden; and Conray 400 (Conray) from Daiichi Seiyaku Co., Tokyo, Japan.

Heparinized venous blood (20 IU heparin/ml) was obtained from the antecubital vein of several healthy adult donors, mixed with balanced salt solution, and layered over a mixture of Ficoll-Conray. Mononuclear cells were separated according to the Boyum method using sterile technique.[10] Granulocytes were separated from the granulocyte-RBC pellet by dextran sedimentation. Following washing, the granulocytes were suspended in TC199 at a final concentration of 1×10^6 cells / 10 μl. Granulocyte viability was greater than 98% by trypan blue exclusion.

The chemoattractant used for these studies was fresh human AB serum.[9] A single sample of fresh serum was obtained from a healthy AB blood group donor and kept frozen at −20°C. Freshly thawed aliquots were used as required.

Agarose plates were prepared as described by Nelson et al[7] with some modifications. Agarose was dissolved in boiling sterile distilled water, cooled, and made up to a solution containing 1% agarose in TC199 supplemented with 10%

果たす微小管集合を損傷する.[4-6] コルヒチンが顆粒球に及ぼす影響を調べたこれまでの研究の多くはBoyden chamber 技法，若しくはその変法を用いており，培養時間は 4 − 5 時間以内であった.[1,3-5]

本研究の目的は，種々の濃度のコルヒチンがヒトの顆粒球の運動性に及ぼす影響を，16時間までの様々な遊走期間において調べることである．アガロースプレート法の変法[7-9]を用いて，不規則遊走と走化性の双方を評価することができた．

材料及び方法

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 (agarose) －半井化学薬品，京都；培養液 TC－199 (TC 199) －日水製薬，東京；仔ウシ胎児血清 (FCS) － Grand Island Biological Co., New York 州 Grand Island 市； コルヒチン－和光化学，大阪； Dextran T 500 (Dextran) － Pharmacia, Sweden, Uppsala 市；プラスティック製ペトリ皿 (60×15㎜) － Falcon, California 州 Oxnard 市； Ficoll 400 (Ficoll) － Pharmacia, Sweden, Uppsala 市； Conray 400 (Conray) －第一製薬，東京．

ヘパリン添加静脈血 (ヘパリン20 IU／ml) を数人の健康な成人の肘前静脈から採取し，平衡化食塩水と混合し，Ficoll-Conray 混合液上に重層した．滅菌技法使用の Boyum 法に従って単核細胞を分離した.[10] 顆粒球は dextran 沈降によって顆粒球－赤血球ペレットから分離した．これを洗浄した後，最終的に 1×10^6 個／1.0μl の濃度になるように TC 199中に懸濁した．顆粒球の生存率をトリパン・ブルー排除検査で測定した結果は98%以上であった．

これらの検査に用いた化学誘引剤は新鮮なヒト AB 血清である.[9] 血液型 AB の健康な成人1人から新鮮血清標本を1回採取し，−20℃で冷凍保存し，必要に応じて一部標本を解凍して使用した．

アガロースプレートは Nelson ら[7]の方法に若干の修正を加えて作製した．アガロースを沸騰した滅菌蒸留水に溶かし，冷却して10% FCS を加えた TC 199中に1%のアガロースを含む液を作った．これに

FCS. Penicillin G, 100 IU/ml and streptomycin, 100μg/ml were added. Colchicine was incorporated into the media of some plates to achieve concentrations of 10^{-7}M or 10^{-5}M. Control plates were prepared without the use of colchicine. The agarose was hardened by refrigeration from 30-60 minutes following which a series of five wells, each 3.0mm in diameter and the edges of which were 4.0mm apart, were cut in a straight line by means of a stainless steel template. The agarose plug in the center of each well was removed by means of gentle suction using a Pasteur pipette.

Ten μl of chemoattractant was placed in the center well, and 10μl of TC199 in each of the outer two wells. To each of the other two wells, 10μl of TC199 containing 1×10^6 granulocytes was added. The plates were incubated at 37°C in a humidified 5% CO_2 incubator. Chemotaxis and random migration were measured by determining the distance from the edge of the well containing the cells to the leading edge of the migrating cells in the direction of the chemoattractant, and control (TC199), respectively, as previously described.[7] Experiments were performed in duplicate and the results averaged as the final end point for each determination. Incubation was continued for 16 hours during which time measurements were made at 2-hour intervals by means of an inverted microscope with an ocular eyepiece grid. Permanent preparations were made at 16 hours by fixation of the plates with Carnoy's solution for 30 minutes, following which the agarose gel was removed and the plates were stained with Wright-Giemsa stain.

In order to be certain that neither the colchicine nor other conditions were altered through prolonged incubation, some plates were preincubated prior to the granulocyte experiments. Agarose plates containing 10^{-7}M colchicine, 10^{-5}M colchicine or no colchicine were placed in the incubator for 16 hours and removed at 2-hour intervals for mock measurements. The agarose of the plate then was hardened by refrigeration for two hours, following which wells were punched, freshly-prepared granulocytes inserted and the usual migration procedure carried out as described above.

RESULTS

Effect of 10^{-7}M and 10^{-5}M Colchicine on

ペニシリンGを100IU／ml，ストレプトマイシンを100μg／ml加えた．コルヒチンを幾つかのプレートの培地に混入し，濃度を10^{-7}Mか10^{-5}Mにした．コルヒチンを用いない対照用プレートも作製した．アガロースは30～60分間冷蔵して固め，ステンレス製型抜きを用いて直径3.0㎜の試料孔を一直線に5個，各孔の端が4㎜間隔になるようにくりぬいた．各孔の中心のアガロース栓を Pasteur ピペットで静かに吸引して取り除いた．

真中の試料孔に10μlの化学誘引剤を入れ，その両側孔2個に10μlの TC 199を入れた．残りの孔2個には1×10^6個の顆粒球を含んだ TC 199を10μl 加えた．プレートを高湿5％ CO_2恒温器中で37℃で培養した．以前に記述したように対照(TC 199)と共に，細胞を入れた試料孔の端から化学誘引剤の方向へ最も遠く遊走した細胞の先端までの距離を測って走化性と不規則遊走を測定した．[7] 実験は2回実施し，その平均値を各実験の最終成績値とした．培養は16時間続け，その間に2時間ごとに接眼レンズグリッド付倒立顕微鏡で測定を行った．16時間後にプレートを Carnoy 液で30分間固定し，アガロース・ゲルをはがし，プレートを Wright-Giemsa 染色して永久的な標本を作った．

長時間にわたる培養中にコルヒチンその他の条件が変化していないことを確認するために，若干のプレートを顆粒球実験を行う前に予備培養した．10^{-7}Mや10^{-5}Mのコルヒチンを含んだりそれを含まないアガロースプレートを恒温器中で16時間培養し，2時間ごとに模擬測定を行った．次にプレートのアガロースを2時間冷蔵して固め，試料孔をくりぬき新鮮な顆粒球を注入して上記の遊走実験を行った．

結　果

10^{-7}及び10^{-5}Mのコルヒチンが走化性に及ぼす影響．

FIGURE 1 EFFECT OF COLCHICINE ON HUMAN GRANULOCYTE CHEMOTAXIS UNDER AGAROSE
図1　ヒト顆粒球のアガロース培地下走化性に及ぼすコルヒチンの影響

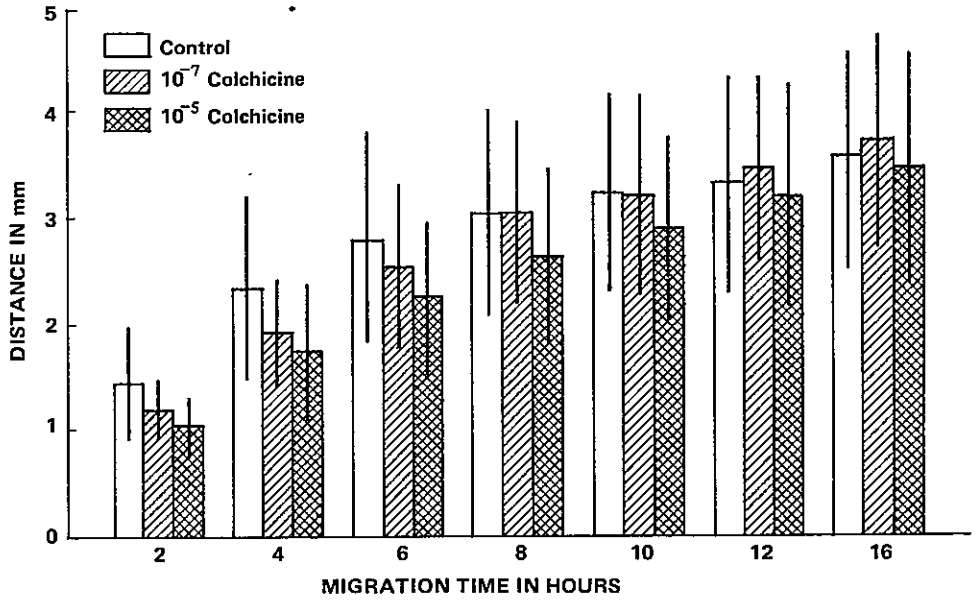

TABLE 1 MEAN PERCENT OF CHEMOTAXIS DISTANCE ACHIEVED BY COLCHICINE-TREATED CELLS COMPARED WITH CONTROLS
表1　コルヒチン処理血球の走化性遊走距離，対照と比較した平均百分率

Colchicine	Hours						
	2	4	6	8	10	12	16
10^{-7}M	79.6†	80.6	90.2	100.1	99.7	101.7	104.4
10^{-5}M	70.8*	73.9	79.5	86.2	88.2	94.3	97.4

†.05＜P＜.10 (borderline境界性)
*P＜.05

Chemotaxis. The effect of 10^{-7}M and 10^{-5}M colchicine on the chemotaxis of granulocytes from 13 individuals is shown in Figure 1 and Table 1. The average distance achieved by the leading front of cells toward the chemoattractant at two hours was reduced in both the 10^{-7}M (.05<P<.1) and 10^{-5}M (P<.05) preparations as compared to the controls. The average migration distances for the 10^{-5}M and 10^{-7}M colchicine-exposed cells were 1.04±0.3mm and 1.17±0.3mm, respectively, in comparison to a control of 1.46±0.5mm. Chemotaxis was about 20%-30% inhibited by these concentrations of colchicine at two hours (Table 1). At four hours and

図1及び表1に，10^{-7}M及び10^{-5}Mのコルヒチンが13人から採取した顆粒球の走化性に及ぼす影響を示した．2時間の時点で化学誘引剤の方向に遊走した血球の最大遊走距離は，対照血球と比較して10^{-7}M (.05＜P＜.1)，10^{-5}M (P＜.05) の双方とも短かかった．10^{-5}M及び10^{-7}Mのコルヒチン処理血球の平均遊走距離はそれぞれ1.04±0.3㎜，1.17±0.3㎜であり，対照値は1.46±0.5㎜であった．2時間の時点では，これらの濃度のコルヒチンによる走化性の抑制は約20%～30%であった(表1)．しかし

FIGURE 2 EFFECT OF COLCHICINE ON HUMAN GRANULOCYTE RANDOM MIGRATION UNDER AGAROSE

図２　ヒト顆粒球のアガロース培地下不規則遊走に及ぼすコルヒチンの影響

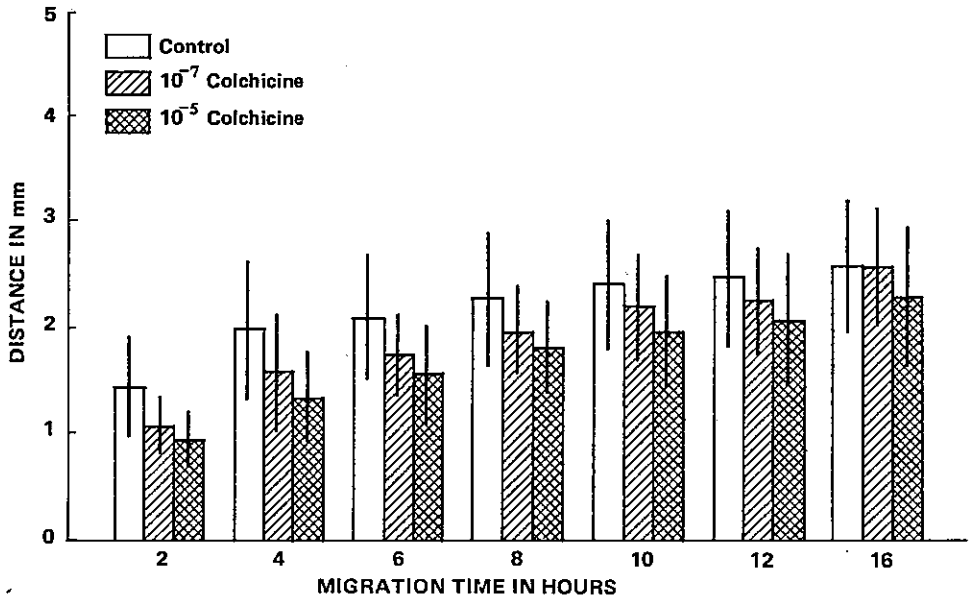

TABLE 2 MEAN PERCENT OF RANDOM MIGRATION DISTANCE ACHIEVED BY COLCHICINE-TREATED CELLS COMPARED WITH CONTROLS

表２　コルヒチン処理血球の不規則遊走距離，対照と比較した平均百分率

Colchicine	Hours						
	2	4	6	8	10	12	16
10^{-7}M	75.4*	80.7	84.0	85.4	91.4	91.7	99.7
10^{-5}M	65.9**	67.6**	75.0*	83.3	81.3	83.7	89.5

*P<.05　**P<.01

thereafter, however, the migration distances for the colchicine-exposed and nonexposed cells were no longer significantly different.

Effect of 10^{-7}M and 10^{-5}M Colchicine on Random Migration. The distance achieved by the leading front of cells toward the control well was recorded at the same 2-hour intervals in each individual as described above for chemotaxis, and the results are shown in Figure 2 and Table 2. At two hours, significant inhibition was observed for the cells exposed to both 10^{-7}M (P<.05) and 10^{-5}M (P<.01) colchicine as compared to the controls. The average migration distances

4時間以降では，コルヒチン処理及び未処理の血球の遊走距離に有意差はなくなった．

10^{-7}M及び10^{-5}Mのコルヒチンが不規則遊走に及ぼす影響．上記の走化性の場合と同様，各標本の対照試料孔への血球の最大遊走距離を２時間ごとに記録し，その結果を図２及び表２に示した．2時間の時点では，対照標本と比較して10^{-7}M（P＜.05）及び10^{-5}M（P＜.01）の双方のコルヒチン処理血球に有意な抑制が見られた．10^{-5}M及び10^{-7}Mの

for the 10^{-5}M and 10^{-7}M colchicine-exposed cells were 0.93±0.3mm and 1.07±0.3mm, respectively, in comparison to a control value of 1.41±0.5mm. Random migration was about 25%-35% inhibited by these concentrations of colchicine at two hours (Table 2). With 10^{-5}M colchicine, significant inhibition was observed at four hours ($P<.01$) and again at six hours ($P<.05$). At eight hours and thereafter, however, the difference was no longer significant. With 10^{-7}M colchicine, significant inhibition was observed only at the first two hours.

Effect of Preincubation of Plates. Preincubated as well as nonpreincubated agarose plates were used to study granulocyte migration in 4 persons, who were chosen at random from among the 13 individuals studied. Measurements of random migration and chemotaxis at 2-hour intervals were nearly identical in all cases, with no significant differences based on type of plate used.

DISCUSSION

Colchicine inhibition of both random migration and chemotaxis was observed to depend on incubation duration. During approximately the first 2-4 hours of incubation significantly shorter distances were achieved by colchicine-exposed cells compared with controls. As the incubation time was increased, however, cells exposed to colchicine "caught up" with controls, and by 8-10 hours or later no significant differences were observed among the preparations. This appeared to result from decreased speed of the colchicine-exposed cells during the early stages of migration. During the first 4-6 hours of incubation, the control cells rapidly out-migrated and then slowed quickly. In contrast, the cells exposed to colchicine migrated more slowly initially, but after 4-8 hours of incubation, the period when nonexposed cells began slowing, the colchicine-exposed cells continued to migrate at a relatively faster rate, allowing them to "catch up" with the nonexposed cells.

Our observations during the first 2-4 hours are consistent with earlier reports of impaired granulocyte motility from colchicine.[1,3-5] Most of the previous studies, however, used incubation periods of 4-5 hours or less, the period during which colchicine effects are most pronounced. It seems likely that had these studies been conducted for longer periods of time, the

コルヒチン処理血球の平均遊走距離はそれぞれ0.93±0.3mm, 1.07±0.3mmであり，対照値は1.41±0.5mmであった．2時間の時点では，これらの濃度のコルヒチンによる不規則遊走の抑制は約25%～35%であった（表2）．10^{-5}Mのコルヒチンを用いた場合，4時間（$P<.01$）と6時間（$P<.05$）の時点でも有意な抑制が認められた．しかし，8時間以降は有意差はなくなった．10^{-7}Mのコルヒチンの場合は，有意な抑制が認められたのは最初の2時間の時点のみであった．

プレートの予備培養の影響． 予備培養したアガロースプレートと予備培養しないものの双方を用いて，13人の調査対象者から無作為抽出した4人の顆粒球遊走を調べた．2時間の時点での不規則遊走及び走化性の測定値はどの標本についてもほぼ等しく，使用したプレートによる有意差はなかった．

考　察

不規則遊走及び走化性の双方に対するコルヒチンの抑制は培養時間によって左右された．約2～4時間の培養では，コルヒチン処理血球の遊走距離は対照と比較して有意に短かかった．しかし，培養時間が長くなるに従って，コルヒチン処理血球は対照標本に「追いつき」，8～10時間以降は有意差は見られなかった．これは，コルヒチン処理血球の遊走速度が，初期の段階で遅くなることによると思われる．培養4～6時間では対照の血球は遊走が速く，その後直ぐに遅くなった．これに対してコルヒチン処理血球は最初の遊走は遅いが，未処理血球の遊走速度が落ち始めるころの培養4～8時間後に相対的に速い速度で遊走を続け，未処理血球に「追いつく」．

初期2～4時間の時点での本研究の観察所見は，コルヒチンによる顆粒球の運動性損傷に関する以前の報告と一致する．[1,3-5] しかし，以前の研究の多くは培養時間が4～5時間以内で，この間はコルヒチンの影響が最も顕著である．したがって，これらの研究が更に長い培養時間を用いていれば，コルヒチン処理

"catching up" effect of the colchicine-exposed cells might also have been observed.

One possible explanation for this colchicine-induced slowing may be that, on the average, both randomly migrating and chemotactically responding cells have a certain amount of energy for locomotion, and that the effect of colchicine is to impair the migration mechanism without significantly affecting the energy supply of the cells. Colchicine-exposed cells, moving slower during the early incubation period and using less energy, would have more energy available for locomotion later, when the energy stores of the cells unexposed to colchicine are more depleted.

Another possible explanation for the disappearance of significant colchicine inhibition was gradual deterioration of colchicine during the lengthy 16-hour incubation period and multiple removal of the plates for measurements. Photoisomerization of colchicine to inactive lumincolchicines is known to occur.[11] This possibility, as well as the possibility of other changes in the media during the experimental procedure, was ruled out by the use of preincubated agarose plates. Results using plates which had been preincubated prior to use were essentially the same as those obtained using nonpreincubated plates.

The results of these studies are consistent with the earlier observation that colchicine inhibition of human granulocyte chemotaxis in vitro is concentration dependent.[3,4] Greater inhibition of both random migration and chemotaxis was observed at 10^{-5}M colchicine than at 10^{-7}M colchicine. With increased colchicine concentration we have observed even greater toxicity. In previous studies[12] using the agarose plate system, we have observed that at 10^{-3}M colchicine relatively fewer granulocytes migrate out from the wells, and for shorter distances, and that at 10^{-2}M, no detectable migration occurs.

The mechanism by which colchicine at concentrations of 10^{-7}M to 10^{-5}M inhibits granulocyte motility is probably related to antitubulin binding and impaired microtubule assembly.[4,5,13,14] Antitubulins such as colchicine have been found to interfere with several granulocyte functions other than motility, however. Adhesiveness,[15] oxygen consumption,[16] and protein and nucleic acid synthesis[13] are also impaired. Most of the

血球の「追いつき」効果が見られたものと思われる.

コルヒチンによって遊走が抑制される一つの理由としては，普通不規則遊走及び走化的反応を共にする血球は運動に対して一定量のエネルギーを有しており，コルヒチンは血球のエネルギー補給に有意に作用することなく，遊走機序を損傷する影響があることが挙げられる．コルヒチン処理血球は培養初期にはゆっくりと遊走し，使用するエネルギーが少なく，未処理血球が有するエネルギーの貯蔵がひどく減じたころの後期の運動に対し，より多くのエネルギー利用ができるのかもしれない.

コルヒチンによる有意な抑制が消滅するもう一つの理由は，16時間の長時間培養の間にコルヒチンが徐々に減少し，測定のためにプレートを何度もはがすことによる．コルヒチンの光異性化によって非活性ルーミンコルヒチンが生じることが知られている.[11] この可能性及び実験過程で培地に起こる他の変化の可能性は，予備培養したアガロースプレートを用いることによって除外された．予備培養したプレートを用いた実験の結果は，予備培養しないプレートの場合と同じであった.

これら研究の結果は，コルヒチンによるヒト顆粒球の試験管内走化性の抑制が，コルヒチンの濃度に依存するという以前の観察所見と一致する.[3,4] コルヒチン濃度10^{-7}Mよりも10^{-5}Mの方が，不規則遊走と走化性の双方に大きな抑制が見られた．コルヒチン濃度が高くなるに従って毒性は大きくなった．アガロースプレート法を用いた以前の研究では，[12] 10^{-3}Mのコルヒチン濃度では試料孔から遊走する顆粒球は比較的少なく，遊走距離も短く，10^{-2}Mでは探知できる遊走は起こらなかった.

10^{-7}Mから10^{-5}Mの濃度のコルヒチンが顆粒球の運動性を抑制する機序は恐らく抗小管結合と微小管集合の損傷に関連しているものと思われる.[4,5,13,14] しかし，コルヒチンのような抗小管体は顆粒球の運動性以外の幾つかの機能を妨げることが発見された．吸着性,[15] 酸素消費[16] 及び蛋白質と核酸の合成[13] も

effects seen in the range of 10^{-7}M to 10^{-5}M probably depend directly or indirectly on microtubules. At these concentrations, the colchicine binding sites are saturated.[17]

Another important observation from these studies was the similarity of the effect produced by colchicine on random migration and chemotaxis. For both types of migration, early inhibition of the colchicine-exposed cells, followed by "catching up" with controls, was observed. This implies that at least in the agarose plate system, the inhibitory mechanism is similar for both types of migration. Several studies[18–20] have shown that randomly migrating neutrophils may be stimulated to increase their speed by substances in their environment (chemokinesis). Malech et al[4] have shown that neutrophils migrating in the absence of chemokinetic substances are uninfluenced by colchicine, whereas a slight effect was shown in their presence. In the agarose system, serum or serum albumin must be added to the medium in order for granulocyte migration to occur.[7] Since both serum and serum albumin are chemokinetic for neutrophils,[19,20] random migration as assayed in this system is actually chemokinesis. The results of the present study agree with those of Malech et al,[4] and indicate that chemokinesis and chemotaxis are both colchicine sensitive processes and, therefore, probably both are microtubule dependent.

The results of the present study support the hypothesis that impairment of neutrophil motility by colchicine may be at least one of the mechanisms responsible for the beneficial effects of the drug in the treatment of gout. Blood and intragranulocyte levels of 10^{-6}M to 10^{-8}M are achieved following therapeutic administration for gout,[21,22] concentrations which are similar to those used to produce the effects seen in the present study and in a previous study by one of us.[23] Studies on the pathogenesis of acute gout have provided evidence that granulocyte motility is intimately related to the development of the acute attack.[24] Phagocytosis of urate crystals, a constant feature of the acute attack, has been shown to result in the generation of factors chemotactic for granulocytes.[25]

In experimentally produced urate arthritis, the accumulation of granulocytes has been found to be necessary for the occurrence of inflammation.[26] Phelps[27] has shown that urate crystals

損傷を受ける．10^{-7}Mから10^{-5}Mの範囲で見られた影響の多くは直接的若しくは間接的に微小管に依存しているようである．これらの濃度においてはコルヒチン結合域は飽和している．[17]

これら研究から得られたもう一つの重要な観察所見は，コルヒチンが不規則遊走と走化性遊走に及ぼす影響が等しかったことである．どちらの遊走についても，初期にはコルヒチン処理血球が抑制を受け，後に対照標本に「追いつく」現象が見られた．これは，少なくともアガロースプレート法においては，抑制機序は不規則遊走と走化性の双方において等しいことを示している．幾つかの研究では，[18–20] 不規則遊走をする好中球が，周囲の物質の刺激によって遊走速度を増すかもしれないことが認められている（化学運動性）．Malechら[4]は，化学運動性をもつ物質の存在しない所で遊走する好中球はコルヒチンの影響を受けず，それが存在する場合にはわずかな影響が見られることを認めた．アガロース法では，顆粒球遊走を起こすためには血清若しくは血清アルブミンを培地に加えなければならない．[7] 血清，血清アルブミンの双方とも好中球に対しては化学運動性をもつので，[19,20] この方法で測定される不規則遊走は実際に化学運動性のものである．本研究の結果はMalechら[4]の研究結果と一致しており，化学運動性と走化性はいずれもコルヒチンに対して感受性が高く，したがって双方とも恐らくは微小管依存性であることを示す．

本研究の結果は，コルヒチンによる好中球の運動性損傷が，痛風治療においてその薬効を上げるような機序の少なくとも一つであるという仮説を裏付けている．痛風の治療にコルヒチンを投与すると，血液及び顆粒球中の濃度が10^{-6}M～10^{-8}Mとなり，[21,22] これは本研究及び著者の一人が行った以前の研究[23]で影響が見られた場合の濃度と同じである．急性痛風の発病の研究によって，顆粒球の運動性が急性発作の発現と緊密に関連しているという証拠が示された．[24] 急性発作の不変の特徴である尿酸塩結晶の食作用によって，顆粒球に対して走化性をもつ因子を生み出すことが認められている．[25]

実験的に起こした尿酸塩関節炎においては，炎症を起こすには顆粒球の累積が必要であることが発見された．[26] Phelps[27]は，尿酸塩結晶は顆粒球に対して

are chemokinetic for granulocytes, and that this stimulation is inhibited by concentrations of colchicine as low as 10^{-8}M. Therefore, substances are present in the lesion of acute gouty arthritis which are both chemokinetic and chemotactic for granulocytes. The severity of an inflammatory reaction probably depends to some extent on the speed with which the inflammatory cells accumulate at and are mobilized within the inflammatory focus. Colchicine impairment of granulocyte chemokinesis and chemotaxis in vitro at concentrations similar to those obtained in vivo provide additional evidence that impaired motility may be an important mechanism whereby it achieves its therapeutic effect.

化学運動性を有しており，この刺激は10^{-8}Mの低濃度のコルヒチンによって抑制されることを示した．したがって急性痛風性関節炎の病巣には，顆粒球に対して化学運動性と走化性の双方を有する物質が存在する．炎症反応の度合いは恐らくある程度は炎症細胞が累積し，炎症病巣内に動員される速度に依存していると思われる．試験管内でのコルヒチンによる顆粒球の化学運動性及び走化性の損傷が，生体内で得られる濃度と同じ濃度で起こるということは，阻害された運動性が治療効果を得るのに重要な機序であり得るという証拠を更に示すものである．

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