

## Mechanism of Action of Colchicine in Acute Urate Crystal-Induced Arthritis

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*J Clin Invest.* 1979;64(3):775-780. <https://doi.org/10.1172/JCI109523>.

### Research Article

Phagocytosis of urate crystals by human or rabbit neutrophils induces the synthesis and release of a glycoprotein, the crystal-induced chemotactic factor (CCF), which is chemotactically active both in vitro and in vivo. It has been proposed that CCF is a prime mediator of the acute gouty attack. Colchicine has been shown to decrease the production and release of this factor in vitro. In these studies, colchicine, at nonleukopenic doses, is shown to abrogate the acute arthritis induced by monosodium urate crystals in rabbits, but to have no effect upon the arthritis induced by the injection of the purified cell-derived chemotactic factor. Serum colchicine levels were 0.48-0.58  $\mu\text{M}$  at 30 min and 0.12-0.3  $\mu\text{M}$  at 90 min after intravenous injection of 0.2 mg/kg colchicine. Peripheral blood polymorphonuclear leukocytes obtained from colchicine-treated animals migrated normally towards a chemotactic stimulus but failed to produce CCF after phagocytosis of monosodium urate crystals. The dialyzed synovial fluid from rabbits injected with microcrystalline sodium urate contained chemotactic activity that was not present when animals were also given intravenous colchicine or injected intra-articularly with the chemotactic factor formyl-methionyl-leucyl-phenylalanine. Furthermore, the synovial fluid from rabbits injected with microcrystalline sodium urate significantly decreased  $^{125}\text{I}$ -CCF binding to neutrophils. The binding of  $^{125}\text{I}$ -CCF to its neutrophil receptor was not significantly reduced by the synovial fluid of colchicine-treated rabbits nor by the [...]

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# Mechanism of Action of Colchicine in Acute Urate Crystal-Induced Arthritis

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**ABSTRACT** Phagocytosis of urate crystals by human or rabbit neutrophils induces the synthesis and release of a glycoprotein, the crystal-induced chemotactic factor (CCF), which is chemotactically active both *in vitro* and *in vivo*. It has been proposed that CCF is a prime mediator of the acute gouty attack. Colchicine has been shown to decrease the production and release of this factor *in vitro*. In these studies, colchicine, at nonleukopenic doses, is shown to abrogate the acute arthritis induced by monosodium urate crystals in rabbits, but to have no effect upon the arthritis induced by the injection of the purified cell-derived chemotactic factor. Serum colchicine levels were 0.48–0.58  $\mu\text{M}$  at 30 min and 0.12–0.3  $\mu\text{M}$  at 90 min after intravenous injection of 0.2 mg/kg colchicine. Peripheral blood polymorphonuclear leukocytes obtained from colchicine-treated animals migrated normally towards a chemotactic stimulus but failed to produce CCF after phagocytosis of monosodium urate crystals. The dialyzed synovial fluid from rabbits injected with microcrystalline sodium urate contained chemotactic activity that was not present when animals were also given intravenous colchicine or injected intra-articularly with the chemotactic factor formyl-methionyl-leucyl-phenylalanine. Furthermore, the synovial fluid from rabbits injected with microcrystalline sodium urate significantly decreased  $^{125}\text{I}$ -CCF binding to neutrophils. The binding of  $^{125}\text{I}$ -CCF to its neutrophil receptor was not significantly reduced by the synovial fluid of colchicine-treated rabbits nor by the synovial fluid of control rabbits injected with the chemotactic factor formyl-methionyl-leucyl-phenylalanine. Colchicine (10 and 0.1  $\mu\text{M}$ ) was shown to have no effect upon the binding of  $^{125}\text{I}$ -CCF to its cell receptor.

Received for publication 11 December 1978 and in revised form 1 May 1979.

## INTRODUCTION

Although colchicine has been used for over a century in the treatment and prevention of gouty arthritis, a complete understanding of its site(s) of action has remained elusive. Colchicine is unique among therapeutic agents used in the treatment of acute arthritis in that its usefulness is generally felt to be relatively limited to this rheumatic disease and that it is not a potent general anti-inflammatory agent (1–3). Experimentally, colchicine has been demonstrated to dramatically abrogate the inflammatory response to urate crystals in humans (4), but to have only weak effects against induced intradermal staphylococcal infection in guinea pig (5). *In vitro*, colchicine and other anti-microtubule agents have been reported to diminish polymorphonuclear leukocyte (PMN)<sup>1</sup> chemotaxis (6, 7) and lysosomal enzyme release (8). However, it seems unlikely that the major *in vivo* pharmacologic action of colchicine is via either of these mechanisms because neither is unique to urate crystal-induced inflammation, and a drug capable of suppressing such PMN functions would be expected to have more general anti-inflammatory effects than colchicine exhibits. The question therefore remains as to the existence of a relatively unique step in the development of acute urate crystal-induced arthritis which is colchicine-sensitive and of secondary importance in other natural and experimental inflammatory conditions.

Evidence suggests that urate crystal-induced arthritis can develop in the absence of complement (9, 10), Hageman factor (11), and kinins (11); we (12, 13), and others (14), have proposed that the initial stimulus in

<sup>1</sup>Abbreviations used in this paper: BSA, bovine serum albumin; CCF, crystal-induced chemotactic factor; FMLP, formyl-methionyl-leucyl-phenylalanine; MSU, microcrystalline sodium urate; PMN, polymorphonuclear leukocyte(s).

the development of the acute gouty attack is the phagocytosis of urate crystals by PMN (and possibly by other phagocytic cells) which leads to the production of a glycoprotein (8,400 mol wt) chemotactic for neutrophils and monocytes. The glycoprotein is not detected if cells are pretreated with actinomycin D or cycloheximide (15, 16), or if cells are exposed only to urate in solution (14). As first reported by Tse and Phelps (17), the chemotactic activity does not appear if the cells are exposed to colchicine at therapeutic levels before their incubation with urate crystals. More recent studies (15) corroborate and extend these findings to demonstrate that the chemotactic glycoprotein is not detectable in the media or subcellular fractions of colchicine-treated cells allowed to phagocytose urate crystals. It should be noted that phagocytosis per se was not impaired by the colchicine treatment in these experiments. Recent work (18) has shown that the intra-articular injection of the purified urate crystal-induced chemotactic factor (CCF) in rabbits produces a profound arthritis that is histologically identical with that produced by urate crystal injection. However, PMN accumulation in the synovial fluid occurs even more rapidly than in urate crystal-induced arthritis, a finding consistent with the circumvention of the early stages of the inflammatory process consisting of crystal phagocytosis, factor synthesis, and release.

This study presents data indicating that the primary mechanism of colchicine action in ameliorating and preventing the acute gouty attack is by inhibiting the production and(or) release of the cell-derived chemotactic factor which normally mediates the inflammatory response to urate crystals.

## METHODS

**Induction of arthritis.** Microcrystalline sodium urate (MSU) (0.5–8 microns in length) was prepared as described by Seegmiller et al. (19). The crystals were heated at 200°C for 1 h to render them pyrogen free (9). 11 female albino rabbits (3–3.5 kg) were injected intravenously with either 0.05, 0.1, or 0.2 mg/kg of colchicine followed 20 min later by the intra-articular injection of 10 mg of MSU suspended in 0.3 ml of isotonic saline in one knee and 0.3 ml isotonic saline alone in the opposite knee. Nine additional rabbits received the intra-articular injections of MSU, but not colchicine, and served as positive controls. Peripheral leukocyte counts were obtained before the colchicine injection, 90 min after, and at the time of sacrifice of the animals, 4 h later. In experiments not presented here, we have found that colchicine at concentrations above 0.2 mg/kg is leukopenic for rabbits and, therefore, higher concentrations were not used in these studies.

In a parallel experiment, eight rabbits were injected with 0.2 mg/kg of colchicine followed by intra-articular injections of 20, 10, or 2 µg of purified CCF in 0.3 ml isotonic saline in one knee and 0.3 ml isotonic saline alone in the opposite knee. The CCF was isolated from rabbit neutrophils allowed to phagocytose MSU as previously described (13). Eight

additional rabbits were injected with the chemotactic factor, but not colchicine, and served as controls. Animals were sacrificed at 90 min after intra-articular injections, and the synovial fluid and synovial membrane were processed as described below. The times chosen to sacrifice the animals injected with MSU or chemotactic factor are those that correspond to the maximal inflammatory response observed in the rabbit model (10, 18).

Two parameters were employed to assess the inflammatory response: histologic evaluation of the synovium, and leukocyte counts in the synovial fluid. The animals were sacrificed, and synovial tissue samples were stained with hematoxylin and eosin. Samples were cultured in broth and blood agar to exclude the possibility of bacterial contamination. Synovial fluid was collected from the open joint of the animals with heparinized plastic pipettes. The histological preparations were evaluated blindly. The leukocyte counts in the synovial fluid were performed immediately with a Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.) (11).

**In vitro chemotactic assay.** Peripheral blood PMN from three rabbits collected immediately preceding and 90 min after intravenous injection of colchicine (0.2 mg/kg) were isolated by a Ficoll (Pharmacia Fine Chemicals Inc., Piscataway, N. J.)-Hypaque (Winthrop Laboratories, New York) centrifugation technique followed by dextran (Pharmacia Fine Chemicals Inc.) sedimentation (20). The ability of the PMN to migrate toward a chemotactic gradient was evaluated by a radioassay that uses Na<sub>2</sub> <sup>51</sup>CrO<sub>4</sub> (New England Nuclear, Boston, Mass.) as previously described (13). Two chemotactic factors were used in the assay, the synthetic peptide formyl-methionyl-leucyl-phenylalanine (FMLP) (a generous gift from Dr. Richard J. Freer, Medical College of Virginia, Richmond, Va.) and CCF isolated as previously described (13). In a separate experiment, the synovial fluid from three rabbits injected intra-articularly with MSU, three injected intra-articularly with MSU and intravenously with 0.2 mg/kg colchicine, and of two additional animals injected intra-articularly with 10 nM FMLP in 0.3 ml of saline were centrifuged, and the supernates were dialyzed and tested for their ability to attract PMN in the Boyden chamber at varying concentrations. All synovial fluid samples were dialyzed before performing the chemotactic radioassay. This was done to remove the tripeptide FMLP from synovial fluids. The glycopeptide CCF is not dialyzable (13).

**Generation of cell-derived chemotactic factor.** The peripheral blood (60 ml) from three rabbits was collected 90 min after the intravenous injection of colchicine (0.2 mg/kg), the PMN were then isolated by Ficoll-Hypaque centrifugation followed by dextran sedimentation and incubated with 5 mg MSU for 45 min at 37°C. The cells were then subjected to the previously described procedure employed to isolate the CCF (13). The isolated material was tested in the chemotactic chambers. The peripheral blood PMN from two rabbits not injected with colchicine were subjected to the same procedure and used as control.

**Determination of serum colchicine levels.** Tritiated colchicine (ring C, methoxy-<sup>3</sup>H, 16.05 Ci/mmol sp act, in benzene:ethanol) was purchased from New England Nuclear. An aliquot of the [<sup>3</sup>H]colchicine was redissolved in an aqueous solution of 500 µg/ml unlabeled colchicine which was then injected into three rabbits intravenously (0.2 mg/kg). At stated times serum samples were obtained, and the radioactivity was determined in 10- to 100-µl aliquots after the addition of 10 ml of Aquasol-2 (New England Nuclear). The radioactive counts per minute were used to calculate the total colchicine concentration in each of the serum samples. One of the rabbits injected with tritiated colchicine was also injected intra-articularly with 10 mg MSU. At the end of the experiment,

the rabbit was sacrificed and synovial fluid radioactivity determined as described above.

**Receptor binding competition assay.** CCF was iodinated as described previously (21). The specific activity of  $^{125}\text{I}$ -CCF was  $39 \mu\text{Ci}/\mu\text{mol}$ . The knee joint of nine rabbits was injected with 3 mg MSU in 0.3 ml saline (six animals) or 10 nM FMLP in 0.3 ml saline (three animals). Three of the rabbits injected with monosodium urate crystals also received intravenous colchicine (0.2 mg/kg). After 3 h, the animals were sacrificed, and the synovial fluid from the joints was removed, pelleted, and diluted with phosphate-buffered saline. In the binding assays,  $1 \times 10^7$  human PMN were incubated at  $37^\circ\text{C}$  in a total volume of 0.5 ml of Hanks' balanced saline solution, pH 7.4, which contained 0.1% bovine serum albumin (BSA) with either  $^{125}\text{I}$ -CCF (final concentration  $0.24 \mu\text{M}$ ) or  $^{125}\text{I}$ -CCF and various concentrations of synovial fluid from the animals injected with either crystals, or FMLP which served as control. We have previously shown that FMLP does not compete with  $^{125}\text{I}$ -CCF for binding sites in the cell membrane (21). After 60 min of incubation, 0.5 ml ice-cold Hanks'-0.1% BSA solution was added, followed by centrifugation in a microfuge (model B, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 8,000 g for 2 min. The supernate was discarded, the pellet was resuspended in Hanks'-0.1% BSA, transferred to another tube, and pelleted again. After removing the supernate, the pellet was counted in a gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.).

In a separate experiment, binding studies of  $^{125}\text{I}$ -CCF to human PMN were performed in the presence of 10 and  $0.1 \mu\text{M}$  of colchicine. The cells were preincubated with colchicine for 20 min at  $37^\circ\text{C}$  before the addition of  $^{125}\text{I}$ -CCF, and the procedure was conducted as described above.

The binding experiments with  $^3\text{H}$ FMLP were performed with the same procedure described for  $^{125}\text{I}$ -CCF except that the buffer used was 0.05 Tris-HCl buffer, pH 7.4, which contained 1 mM of  $\text{MgCl}_2$  (22). The chemotactic peptide was purchased from New England Nuclear, and had a  $57 \text{mCi}/\mu\text{mol}$  sp act.

## RESULTS

An inflammatory response manifested by intense leukocytic accumulation in the synovial fluid and synovial membrane was observed after the injection of MSU into the knee joint of rabbits. The differential count in the synovial fluid at 4 h showed 95% neutrophils and 5% mononuclear cells. The synovial fluid obtained from saline control joints showed only minimal leukocytosis. The histological evaluation of the synovial membranes from joints injected with crystals demonstrated an inflammatory infiltrate composed of mononuclear cells and neutrophils (figure not shown). When the animals were injected intravenously with colchicine, before the intra-articular injection of crystals, a dose-related decrease in the inflammatory response was noted, as measured by the number of leukocytes present in the synovial fluid as well as the degree of synovial membrane infiltration. The maximal effect was noted with 0.2 mg/kg colchicine (Fig. 1). No peripheral leukopenia at this colchicine concentration was noted. Colchicine, when injected at concentrations higher than 0.2 mg/kg was noted to be leukopenic for the rabbits.

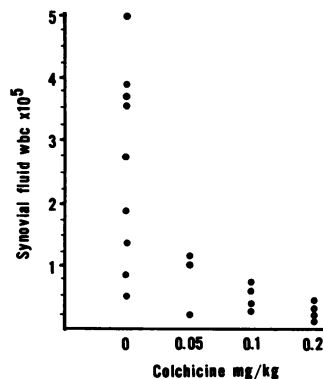


FIGURE 1 Effect of colchicine on MSU-induced arthritis. Synovial fluid leukocyte (wbc) count of rabbits injected intra-articularly with MSU and intravenously with varying concentrations of colchicine. Analysis of variance (one way anova):  $F_{3,16} = 6.01$ ,  $P < 0.01$ . Multiple comparison (Scheffe's test):  $P < 0.05$  for group of rabbits not injected with colchicine (control) vs. group of rabbits injected with 0.05 mg/kg colchicine, and  $P < 0.01$  for the control group vs. group injected with 0.1 or 0.2 mg/kg colchicine. Values represent total leukocyte counts present in the synovial fluid.

When the rabbits were injected with 2, 10, or  $20 \mu\text{g}$  of the CCF, an inflammatory response indistinguishable from that induced by crystals was noted. Pretreatment with colchicine, however, had no effect upon the inflammatory reaction in response to the purified factor (Table I).

The peripheral blood PMN from three rabbits, collected immediately preceding and 90 min after the administration of colchicine, were tested for their ability to migrate towards a chemotactic factor gradient and to generate CCF in vitro after phagocytosis of urate crystals. Although no difference in the chemotactic behavior of the cells was noted when challenged with FMLP or CCF in the Boyden chamber (Table II), the cells treated in vivo with colchicine failed to generate chemotactic activity after phagocytosis of urate crystal in vitro. Their chromatographic fractions gave a chemotactic index of  $0.07 \pm 0.01$  compared with control of  $5.9 \pm 1.0$  obtained from cells not treated in vivo with colchicine.

The synovial fluid obtained from rabbits injected intra-articularly with MSU was tested for its ability to attract human neutrophils in vitro (Table III) and to compete with human  $^{125}\text{I}$ -CCF for binding sites present on the human neutrophils (Table IV). The synovial fluid samples were dialyzed before the chemotactic assays. In the binding experiments, the synovial fluid samples were not dialyzed. The synovial fluid from rabbits injected intra-articularly with MSU was shown to attract human neutrophils at dilutions of 1:5–1:20 and to decrease  $^{125}\text{I}$ -CCF binding to human neutrophils. In contrast, the synovial fluid of colchicine-treated rabbits injected with MSU failed to either

**TABLE I**  
*Effect of Pretreatment with Colchicine on Synovial Fluid Leukocytosis Induced by CCF*

Pretreatment	Intra-articular injection					
	2 µg CCF		Saline		20 µg CCF	
	leukocytes/ mm <sup>3</sup>	leukocytes/ mm <sup>3</sup>	leukocytes/ mm <sup>3</sup>	leukocytes/ mm <sup>3</sup>	leukocytes/ mm <sup>3</sup>	leukocytes/ mm <sup>3</sup>
Colchicine, 0.2 mg/kg	12,561	3,818	31,429	3,178	151,272	2,832
Colchicine, 0.2 mg/kg	10,866	2,319	45,980	2,299	106,590	1,071
Colchicine, 0.2 mg/kg			49,310	3,310	149,630	3,178
None	9,532	2,045	21,318	1,071	159,440	2,972
None	1,014	3,819	50,426	2,521	116,430	4,299
None			45,010	3,050	123,740	2,299

Each pair of numbers (CCF vs. Saline) is derived from single rabbits, one joint injected with CCF in saline and the contralateral one with saline alone. The average peripheral leukocyte count  $\pm$ SD before intravenous colchicine injection was  $6,878 \pm 1,900$ , and at the time the animals were sacrificed was  $6,717 \pm 2,426$ .

significantly attract neutrophils in the Boyden-chamber assay or to appreciably decrease <sup>125</sup>I-CCF binding to its cell receptor. Similarly, the synovial fluid of rabbits injected intra-articularly with FMLP, used as control material, failed to attract neutrophils (after dialysis) or to significantly decrease <sup>125</sup>I-CCF binding to neutrophils. In a separate experiment, the synovial fluid of two rabbits injected intra-articularly with MSU and one injected intravenously also with colchicine were tested for their ability to alter [<sup>3</sup>H]FMLP binding to human neutrophils. No significant decrease in the binding of [<sup>3</sup>H]FMLP to the cells was noted (results not shown).

In experiments designed to investigate the possible effect of colchicine upon <sup>125</sup>I-CCF binding to human neutrophils, the binding studies were performed employing cells preincubated with the drug at concentrations of 10 and 0.1 µM. Colchicine failed to alter the binding of <sup>125</sup>I-CCF to human neutrophils. The specific counts per minute bound to the cells in the absence of colchicine were  $346 \pm 24$  SD; in the presence of colchicine at a concentration of 10 µM,  $336 \pm 20$  SD; and with colchicine present at a concentration of 0.1 µM,  $332 \pm 15$  SD.

**TABLE II**  
*Chemotaxis of Rabbit Peripheral Blood PMN Treated In Vivo with Colchicine*

PMN obtained from rabbits	Chemotactic index	
	FMLP	CCF
Before colchicine injection	7.9 $\pm$ 0.6	6.2 $\pm$ 0.9
90 min after colchicine injection	8.3 $\pm$ 0.2	6.1 $\pm$ 0.7

Results expressed as the mean and SD of three experiments performed in triplicate. Cells were challenged with either 1 nM FMLP or 20 µg CCF.

The serum levels of colchicine were measured in three rabbits. The concentration range obtained in the animals were as follows: at 15 min after injection, the colchicine levels were 0.83–4 µM; at 30 min, the levels had decreased to 0.48–0.58 µM and continued to decline slowly throughout the duration of the experiment (Fig. 2). One of the rabbits was also injected with MSU (10 mg) intra-articularly, and the colchicine levels measured in its synovial fluid was found to be

**TABLE III**  
*Chemotactic Activity of Dialyzed Synovial Fluid from Rabbits Injected Intra-articularly with MSU Crystals*

Rabbit synovial fluid	Chemotactic index
	Mean $\pm$ SD
MSU injected intra-articularly	
Dilutions	
1:5	5.7 $\pm$ 1.2
1:10	4.6 $\pm$ 0.5
1:20	3.5 $\pm$ 0.1
MSU injected intra-articularly and colchicine intravenously	
Dilutions	
1:5	0.4 $\pm$ 0.1
1:20	0.3 $\pm$ 0.4
FMLP injected intra-articularly (control)	
Dilutions	
1:5	0.8 $\pm$ 0.5
1:20	0.2 $\pm$ 0.2

The synovial fluid was diluted to a total volume of 1 ml with Hanks' balanced saline solution. The number of leukocytes present in the synovial fluid in rabbits injected with crystals or FMLP was alike. Numbers represent the mean and standard deviation of three experiments for the rabbits injected with crystals and two for those injected with FMLP. All experiments were performed in triplicate.

**TABLE IV**  
*Inhibition of <sup>125</sup>I-CCF Binding to Human Neutrophils by Synovial Fluid from Rabbits Injected with MSU or with the Chemotactic Factor FMLP*

Neutrophils injected with	Percent decrease in <sup>125</sup> I-CCF binding by synovial fluid from rabbits injected with		
	MSU		FMLP†
	No colchicine§	Colchicine	
<sup>125</sup> I-CCF + 10 μl synovial fluid	18.2±4.6	0	4.4±1.6
<sup>125</sup> I-CCF + 30 μl synovial fluid	68.9±7.8	5.6±1.5	8.2±0.8

100% binding for <sup>125</sup>I-CCF was 3 pmol bound to 10<sup>7</sup> cells. The total volume of the mixture was 0.5 ml. Calculations from the binding-competition curve for <sup>125</sup>I-CCF and unlabeled CCF (23) indicated that 6.8 nmol of unlabeled CCF is required to abolish binding of 3 pmol of <sup>125</sup>I-CCF to the cells. Values represent the mean percent decrease in binding ±SD of three experiments; each performed in quadruplicate. \* 10 mg in 0.3 ml of saline.

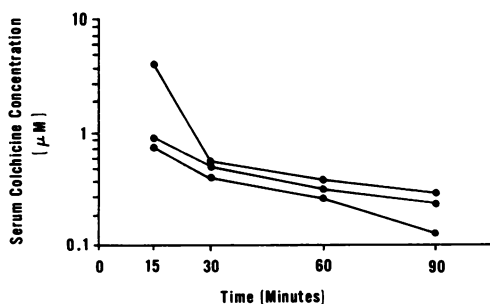
† 1 nM.

§ 0.2 mg/kg.

0.22 μM at 90 min; the plasma level of colchicine in the same animal at 90 min was 0.25 μM (Fig. 2).

## DISCUSSION

This study demonstrates that colchicine, at nonleukopenic doses, can ameliorate, in a dose-dependent manner, the acute inflammatory response in rabbits induced by urate crystals. However, the drug is ineffective in preventing the accumulation of inflammatory cells in the synovial fluid and synovium when purified CCF, a presumed major mediator of acute gouty arthritis, is injected into the joint cavity. The inflammatory response in the animals injected intra-articularly with CCF was identical in control animals and those pretreated with colchicine, which indicates that



**FIGURE 2** Rabbit serum levels of colchicine after the intravenous injection of [<sup>3</sup>H]colchicine. Each line represents one rabbit.

neutrophil migration per se was not effectively altered by colchicine *in vivo* at the concentrations tested. This is directly supported by the *in vitro* chemotaxis study in which cells obtained from colchicine-treated animals behaved no differently in their chemotactic response toward two different chemotactic factors or in their ability to phagocytose crystals. It is of interest to note that the neutrophil functions of phagocytosis and chemotaxis have been reported to be normal in patients with familial Mediterranean fever, who were treated prophylactically with 0.6–1.8 mg/d of colchicine orally (23, 24).

It seems likely that the anti-inflammatory effect of colchicine is directed towards an early step in the development of the inflammatory response induced by urate crystals. *In vitro* studies (15, 17) have shown that colchicine-treated cells can phagocytose MSU crystals, a prerequisite for the appearance of chemotactic activity (25), but the release and/or granule localization of the factor is prevented (15, 17). Likewise, in this study the peripheral blood neutrophils obtained from colchicine-treated rabbits, although able to phagocytose crystals *in vitro*, failed to show detectable chemotactic-factor activity in chromatographic cell fractions. These results may suggest a direct or indirect effect of colchicine on the synthetic apparatus of the cell, an effect reported in other cell systems (26). Selective effects of colchicine on protein secretion and intracellular localization have also been reported (8, 27).

Chemotactic activity was demonstrated to be present in the dialyzed synovial fluid of animals injected intra-articularly with MSU, but only minimal activity was demonstrated in the dialyzed synovial fluid of rabbits pretreated with colchicine or in the dialyzed synovial fluid of control rabbits injected intra-articularly with FMLP. When the synovial fluid of animals injected intra-articularly with MSU was incubated with <sup>125</sup>I-CCF and cells, a dose-related decrease of <sup>125</sup>I-CCF binding to the cells was noted. This decrease in binding was markedly reduced when the synovial fluid of colchicine-treated rabbits was tested, or when the synovial fluid obtained from rabbits injected with FMLP intra-articularly was incubated with <sup>125</sup>I-CCF and cells. Colchicine, at concentrations of 10 and 0.1 μM, did not alter the binding of <sup>125</sup>I-CCF to its cell receptor. In a separate control experiment, the synovial fluid of animals injected with MSU was shown not to affect [<sup>3</sup>H]-FMLP binding to cells. We have previously shown that FMLP and CCF bind to different receptors in the neutrophil (21). The data summarized above would indicate the appearance of CCF *in vivo* in the synovial fluid of the animals injected with MSU. The presence of a chemotactic, stereochemically related substance(s) able to bind to the CCF receptor on the neutrophil and present in the synovial fluid of MSU-injected rabbits, but not in the controls, would appear unlikely.

Because the control synovial fluid obtained from animals injected with FMLP contained a similar number of cells before they were removed before the assay, the possibility of neutrophil-derived peptidases being responsible for lowering  $^{125}\text{I}$ -CCF binding relative to control levels is also unlikely. Furthermore, the synovial fluid from MSU-injected rabbits did not lower the binding of  $^3\text{H}$ FMLP to the cells.

The serum level of colchicine at 30 min was  $\cong 0.1 \mu\text{M}$  and remained so during the duration of the experiment. This colchicine concentration, which approximates that obtainable in man with therapeutic doses of colchicine (2), has been reported to impair the *in vitro* appearance of CCF after interaction of PMN with MSU (12, 15, 17).

In summary, data are presented which support the postulate that the therapeutic efficacy of colchicine in gout and experimental urate crystal-induced arthritis is related to the suppression of chemotactic factor generation and release by the neutrophil. Presumably, colchicine is not a more potent general anti-inflammatory agent because in many other inflammatory states, in contradistinction to crystal-induced arthritis, the neutrophil-derived chemotactic factor is not a major mediator, and therefore its suppression by colchicine only modestly abrogates the inflammatory process. Our data also demonstrate the presence of a chemotactic factor with similar, if not identical, characteristics than human CCF in synovial fluid of rabbits injected with MSU.

#### ACKNOWLEDGMENTS

We wish to thank Mr. Steven Disper and Mrs. Vanita Venugopal for their technical assistance, and Ms. Gerlean Smith for secretarial help.

This investigation was supported by U. S. Public Health Service grant AM-19349 and by an Arthritis Foundation Clinical Research Center grant.

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