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Mechanism of Action of Colchicine. V. Neutrophil Adherence and Phagocytosis in Patients with Acute Gout Treated with Colchicine[1]

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ABSTRACT

The phagocytic activity and adherence of polymorphonuclear leukocytes in 13 patients with acute gouty arthritis were measured before and after a 1-week course of colchicine therapy. Normal subjects were used as controls. Both the phagocytic capacity and the phagocytic rate of polymorphonuclear leukocytes were found to be significantly reduced after colchicine treatment. No change in the adhesiveness of these cells were observed.

Although it is generally believed that colchicine exerts its therapeutic effect in gout by modifying functions of PMNs, perhaps *via* its action on the microtubules (Malawista, 1968), there is no direct evidence supporting this view. Experiments *in vitro* have shown that colchicine can modify several functions of PMNs such as phagocytosis (Chang, 1968; Lehrer, 1973), lysosomal degranulation (Malawista, 1975; Zurier *et al.*, 1973), chemokinesis (Valerius, 1978), adherence (Penny *et al.*, 1966), capping of membrane receptors (Vassalli and Silverstein, 1977), chemotaxis (Borel, 1973) and the release of urate-crystal-induced chemotactic factor (Spilberg *et al.*, 1979). The significance of these *in vitro* demonstrations of cellular effects in regard to *anti*gout activity has been questioned (Wallace, 1975) because the threshold concentrations of colchicine at which suppressive activities were noted in most of the *in vitro* systems used for measuring the above mentioned functions of leukocytes far exceeded the blood levels demonstrated to exist *in vivo* after oral or i.v. administration of colchicine at therapeutic doses (Wallace and Ertel, 1973). Although a reasonable counter argument can be made that plasma levels may not accurately reflect concententrations of colchicine bound to the cells or incorporated intracellularly and that the effective dosage depends upon both the concentration of colchicine and the duration of exposure (which was usually very short in the *in vitro* systems employed), it is clear that, in this instance, *in vitro* experiments cannot provide definitive answers regarding *in vivo* actions of colchicine.

Very few *in vivo* studies on the cellular effects of colchicine have been reported. Spilberg and co-workers (1976, 1977, 1979) showed that colchicine administered to rabbits at nonleukopenic doses inhibited the synthesis and release of a cell-derived chemotactic factor from PMNs. MacGregor (1976) reported that the average value for the adherence of PMNs of five normal volunteers was reduced 29% after the administration of a single oral dose of colchicine (1.8 mg). To our knowledge there has been no report of studies concerning the effect of colchicine treatment, in humans or in experimental animals, on the phagocytic activity of PMNs.

Ideally, experiments aimed at elucidating the mechanisms of colchicine action in gout should be conducted not only *in vivo* but also in patients with gouty arthritis. Urate-crystal-induced arthritis in animals, at least the dog and the rat, does not entirely mimic the human disease (Chang and Malawista, 1976; Webster *et al.*, 1972). Colchicine has been shown to suppress urate-crystal-induced canine joint inflammation at leukopenic doses only, but such large doses are not necessary in acute gouty arthritis. Studies in normal human volunteers also may be misleading because leukocytes in normal subjects may not respond to colchicine in the same manner or to the same degree as those in gouty patients. It is well known that colchicine is most effective when administered during the early stage of a gouty attack. In human gouty subjects given intra-articular urate crystals, the effect of pretreatment with colchicine was best demonstrated in patients who, in control studies, showed the greatest clinical inflammatory responses or greatest leukocytosis in synovial fluid (Malawista and Seegmiller, 1965). The augmented response in some gouty subjects is unexplained, but could be related to the fact that the inflammatory process *per se* modifies functions of the PMN by generation of humoral

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ABBREVIATION: PMN, polymorphonuclear leukocyte.

factors that augment PMN adherence, enhance chemotaxis or stimulate release of lysosomal enzymes (Becker *et al.*, 1974; MacGregor, 1976). This report presents the results of a study on the phagocytic activity and adherence of PMNs in 13 patients with acute gouty arthritis before and after colchicine therapy.

Materials and Methods

Patient population. All 13 patients (male) with acute gouty arthritis had acute involvement of at least one joint of less that 48 hr duration; abrupt onset of excruciating pain in the involved joint accompanied by tenderness, redness and heat; and urate crystals in the synovial fluid shown by polarizing microscopy. Each patient was given 0.6 mg of colchicine orally every 2 hr until toxic symptoms appeared or until a total dose of 6.0 mg over a 24-hr period was reached, followed by daily oral doses of 1.8 mg of colchicine for 1 week. Blood samples were collected just before the initiation of colchicine and then at 1 week after the initiation of therapy, at approximately 18 hr after the last dose. All 13 patients responded to treatment within 48 hr.

Materials. [^{3}H]Uridine (specific activity, 40–60 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Scrubbed nylon fiber (3 denier, 03.81 cm, type 200) was purchased from Fenwall Laboratories (Deerfield, Il). Tuberculin syringes were purchased from Becton-Dickinson & Co. (Rutherford, NJ). One of the authors (E. Dellaverde) donated the blood for the fresh AB serum. Colchicine was purchased from K and K Laboratories (Plainview, NY).

Measurement of phagocytosis. PMNs were isolated and purified from fresh peripheral blood according to the procedure of Boyum (1968). Phagocytic activity was determined by a modification of the method of Yamamura *et al.* (1977) and of Yamamura and Valdimarsson (1978), which was based on the inhibition of [^{3}H]uridine incorporation into *Candida parapsilosis* (yeast). Assays were performed as follows: a suspension of yeast cells (5×10^5) and PMNs (5×10^4) in 0.5 ml of culture medium (RPMI-1640 containing 2.5% serum) was placed in a polypropylene tube (17 × 100 mm) and rotated at 37°C for 5, 10, 20, 30 or 45 min unless otherwise indicated. At the end of the incubation period, 0.35 ml of medium (RPMI-1640, prewarmed to 37°C) containing EDTA (1×10^{-3} M) was added to stop phagocytosis. Triplicate 0.17-ml aliquots were transferred to microtiter wells to which 0.4 μCi of [^{3}H] uridine had previously been added and the microtiter plates were incubated for a further 60 min at 37°C. The cells were then collected onto glass fiber filter paper by a Skatron Multiple Harvester, the dried discs placed in scintillation vials, covered with 3 ml of scintillation fluid and counted in a Searle liquid scintillation spectrometer, model Mark I (6860). Human PMNs phagocytized *C. parapsilosis* rapidly during the first 30 min of incubation and the process was virtually completed by 45 min. The suppression of [^{3}H]uridine uptake measured at 45 min and expressed as a percentage (which is proportional to phagocytosis) was therefore termed "phagocytic capacity."

Phagocytic capacity =

$$\left(1 - \frac{\text{[}^3\text{H]uridine (cpm) uptake by yeast in the presence of PMNs}}{\text{[}^3\text{H]uridine (cpm) uptake by yeast in the absence of PMNs}}\right) \times 100$$

The slope of the percent inhibition-time regression line covering the first 30 min was taken as the "phagocytic rate."

Measurement of leukocyte adhesiveness. The adhesiveness of PMNs was determined by a procedure fashioned after two reported methods (MacGregor *et al.*, 1974; Stecher and Chinea, 1978). Scrubbed nylon fiber, 30 mg (3 denier, 3.81 cm, type 200, Fenwall Laboratories), was packed into the barrel of a 1.0-ml plastic tuberculin syringe to a density of approximately 300 mg of fiber per ml. A 0.5 ml volume of whole heparinized blood (14 U of heparin per ml) was introduced into the fiber column and allowed to drain through the column by gravity at room temperature and the effluent was collected. The number of PMNs in the effluent was counted in a Neubauer hemocytometer. Adherence, expressed as a percentage, was calculated according to the following equation:

$$\% \text{ Adherence} = \left(1 - \frac{\text{PMN/ml of effluent}}{\text{PMN/ml of whole blood}}\right) \times 100$$

Results

Phagocytosis of yeast by PMNs as measured by supression of [^{3}H]uridine uptake. The kinetics of phagocytosis of yeast at 37°C, reflected by decreases in [^{3}H]uridine uptake, are shown in figure 1. The ingestion of yeast was rapid during the first 30 min. This time course for phagocytosis corresponds to that determined by other methods (Cohn and Mores, 1959; Sbarra and Karnovsky, 1959; Chang, 1969). When a constant number of yeast cells (5×10^5) was incubated with varying numbers of PMNs at 37°C for 45 min, the reductions of [^{3}H] uridine uptake observed, expressed as percentages of the control value (yeast alone), were proportional to the number of PMNs present in the culture system (fig. 2).

The phagocytic capacities and phagocytic rates of PMNs of normal human volunteers. The phagocytosis of yeast cells by human PMNs at 37°C essentially reached a maximum after 30 min of incubation. The suppression of [^{3}H] uridine uptake, determined at 45 min after the initiation of incubation of yeast cells (5×10^5) and PMNs (5×10^4), expressed as a percentage of the control value obtained when 5×10^5 yeast cells were incubated alone in the presence of [^{3}H]uridine, was therefore defined as phagocytic capacity. To determine the phagocytic rate, PMNs (5×10^4) and yeast cells (5×10^5) were incubated at 37°C for 0, 5, 10, 20 and 30 min before exposure to [^{3}H]uridine. The reductions of [^{3}H]uridine uptake were plotted against time. The slope of the regression line of the percent suppression *vs.* time represents the rate of phagocytosis.

The phagocytic capacity of PMNs of 20 normal individuals ranged from 30 to 65. The phagocytic rate of PMNs of these same individuals also varied widely (1.0–2.4). However, with any given individual, there was little difference ($P > .2$) between

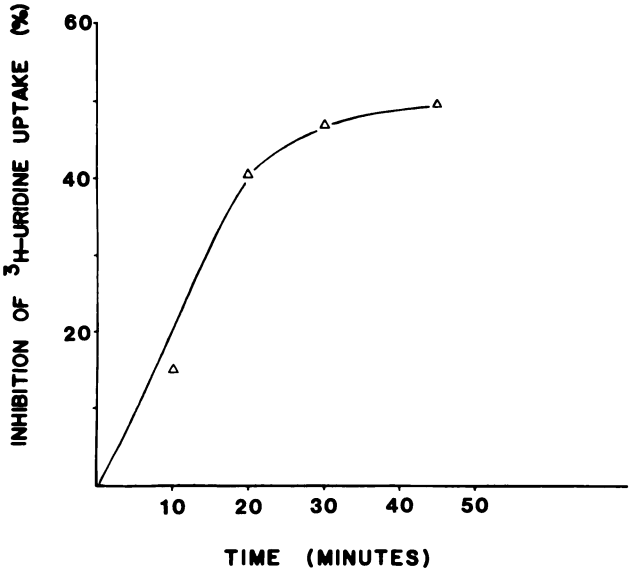

Fig. 1. Kinetics of inhibition of [^{3}H]uridine incorporation into *C. parapsilosis* (5×10^5) by human PMNs (5×10^4) at 37°C.

values of phagocytic capacity or phagocytic rate determined 1 week apart (tables 1 and 2).

Effect of colchicine treatment on the phagocytic capacity and the phagocytic rate of PMNs. Eleven patients with acute gouty arthritis were studied. Each patient was given 0.6 mg of colchicine orally every 2 hours until toxic symptoms appeared or until a total dose of 6.0 mg over a 24-hr period was reached, followed by daily oral doses of 1.8 mg of colchicine for 1 week. Blood samples were collected just before the initiation of colchicine therapy and again on the 7th day after the initiation of therapy at approximately 18 hr after the last oral dose.

The mean values of phagocytic capacity (table 1) and the phagocytic rate (table 2) of PMNs obtained from patients 1 week after the initiation of colchicine therapy were both significantly lower than the corresponding pretreatment values ($P < .01$). Although the pretreatment values of phagocytic rate for patients appeared to be higher and the post-treatment values lower than those of normal individuals, the observed differences were not statistically significant.

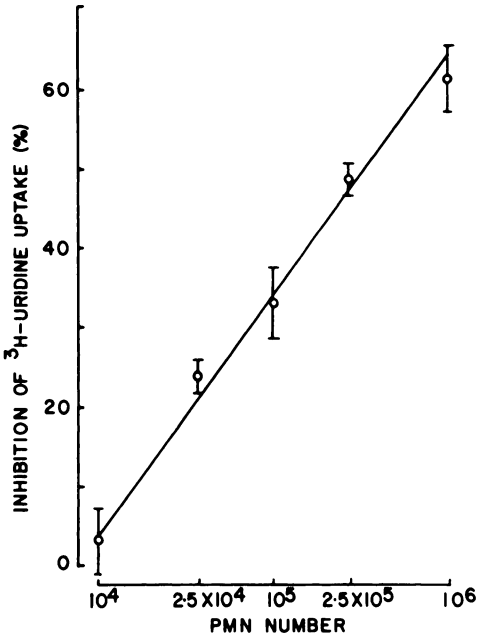

Fig. 2. Suppression of [^{3}H]uridine incorporation into *C. parapsilosis* (5×10^5) as a function of the number of available human PMNs.

The change in phagocytic capacity after colchicine treatment appeared to be due to changes intrinsic to the cells because similar results were obtained when phagocytic capacities were determined in the presence of AB serum instead of fresh autologous serum (fig. 3).

Adhesiveness of PMNs as measured by nylon fiber column. The adhesiveness of PMNs was measured using nylon fiber columns. When the number of PMNs introduced into the column was kept constant, the number of PMNs retained by the column increased progressively with increasing weight of nylon fiber used to pack the column (fig. 4). When the amount of fiber was kept constant, the proportion of PMNs retained by the column decreased progressively with increasing numbers of cells introduced into the column (fig. 5) as expected for a saturable system. Suspensions of aggregated cells (induced by Con A) did not show this phenomenon, indicating that the procedure measures adherence rather than trapping (fig. 5).

Adhesiveness of PMNs of normal human volunteers and of patients with acute gouty arthritis treated with colchicine. The adhesiveness of PMNs of 20 normal individuals was found to vary over a wide range (42–84%). However, the values obtained 1 week apart for any given normal individual were quite consistent in the seven individuals so studied (table 3). Ten patients with acute gouty arthritis were treated with colchicine for 1 week as described previously. Blood samples were taken just before the initiation of colchicine therapy and again on the 7th day after the initiation of therapy at approximately 18 hr after the last oral dose. No change was observed after colchicine therapy (table 3).

Discussion

In most of the studies reported in the literature on neutrophil phagocytosis, the phagocytic activity was measured after an incubation period of 30 min to 2 hr. Such measurements represent the phagocytic capacity of the cells because neutrophil phagocytosis under standard conditions reaches maximum in about 30 min regardless of the method of measurement used or

TABLE 1

Phagocytic capacity of PMNs from patients with acute gouty arthritis before and after colchicine treatment and of PMNs from normal individuals determined on two occasions 1 week apart

Normal Controls			Patients[a]		
Subjects	Phagocytic capacity		Subjects	Phagocytic capacity	
	Day 0	Day 7		Day 0	Day 7
C. E.	56.4	53.0	J. B.	67.8	31.0
M. H.	33.2	33.8	R. B.	59.4	48.7
P. C.	50.1	54.3	N. C.	43.2	33.6
K. L.	53.6	49.7	S. D.	61.6	28.2
E. D.	53.0	50.8	H. L.	36.8	31.5
B. H.	64.3	58.8	T. F.	40.8	24.4
P. P.	50.3	54.6	A. D.	48.0	22.3
C. M.	60.5	57.4	J. S.	59.2	41.7
			A. D.	57.3	54.4
			N. D.	52.8	43.7
			A. A.	47.8	48.8
Mean ± S.E.	52.68 ± 3.28	51.55 ± 2.75	Mean ± S.E.	52.25 ± 2.93	37.12 ± 3.27**

[a] Patients were treated with colchicine as described under "Methods." Blood samples were obtained just before the initiation of therapy (day 0) and then at 1 week after the initiation of therapy (day 7).

** Significantly different from the mean of pretreatment (day 0) values ($P < .01$, Student's t test).

TABLE 2

Phagocytic rate of PMNs from patients with acute gouty arthritis before and after colchicine treatment and of PMNs from normal individuals determined on two occasions 1 week apart

Normal Controls			Patients[a]		
Subjects	Phagocytic rate		Subjects	Phagocytic rate	
	Day 0	Day 7		Day 0	Day 7
C. E.	1.89	1.59	J. B.	3.01	1.28
M. H.	0.99	1.08	R. B.	2.61	2.60
P. C.	1.90	1.98	N. C.	2.10	1.41
K. L.	2.37	1.87	S. D.	2.18	1.27
E. D.	2.16	2.68	H. L.	1.47	1.27
B. H.	1.97	1.98	T. F.	0.98	0.97
P. P.	1.65	1.84	A. D.	2.27	0.67
S. D.	1.60	1.30	J. S.	2.18	1.11
			N. D.	2.59	1.68
			A. A.	2.70	1.89
			C. B.	2.92	1.80
Mean ± S.E.	1.82 ± 0.15	1.79 ± 0.17	Mean ± S.E.	2.27 ± 0.18	1.45 ± 0.16**

[a] Patients were treated with colchicine as described under "Methods." Blood samples were obtained just before the initiation of therapy (day 0) and then at 1 week after the initiation of therapy (day 7).

** Significantly different from the mean of pretreatment (day 0) values.

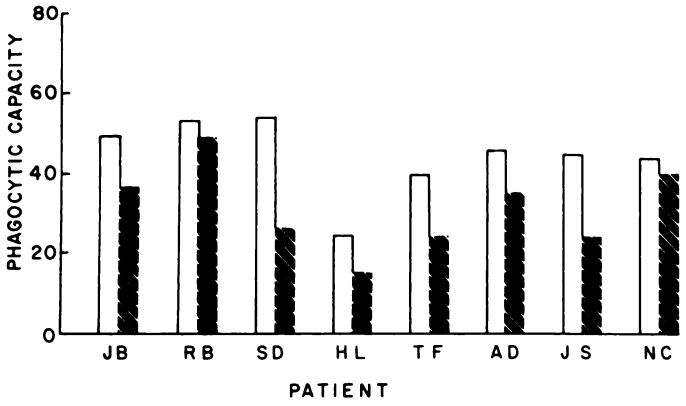

Fig. 3. Effect of colchicine treatment (see text) in patients with acute gouty arthritis on the phagocytic capacity of PMNs determined in the presence of AB serum. Post-treatment blood samples (darkened bars) were obtained 1 week after the initiation of colchicine therapy.

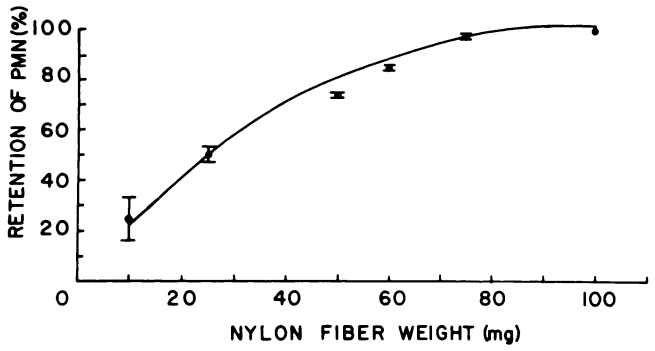

Fig. 4. Column retention of PMNs as a function of the amount of nylon fiber in the column.

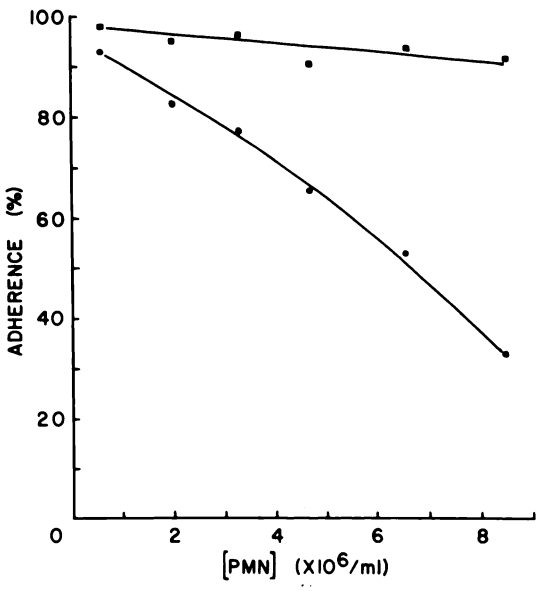

Fig. 5. Retention of PMNs by nylon fiber (10 mg) column as a function of the number of leukocytes. Comparison between aggregated (Con A treated) (■) and nonaggregated (●) cells suspended in phosphate buffer.

the type of particles employed. To determine possible effects of colchicine on the phagocytic activity of neutrophils, it is important to measure not only the phagocytic capacity but also the rate of phagocytosis which may be reduced or accelerated without any apparent change of the former. The determination of phagocytic rate involves multiple measurement at several time points. Considering the relatively small samples of blood that can be obtained from patients, the method chosen for the investigation of possible colchicine-induced changes in phagocytic activity must, therefore, require relatively few cells, not be too cumbersome in operation and must be sensitive, quantitative and reproducible. The only method in the literature which seemed potentially capable of meeting these stringent requirements was that reported by Yamamura et al. (1977) and Yamamura and Valdimarsson (1978), which measures the suppression by leukocytes of [^{3}H]uridine incorporation into *C. parasilosis* (yeast). The method was based on the observations that 1) there is a linear correlation between uridine incorporation and the number of yeast cells; 2) leukocytes do not incorporate significant amounts or uridine in short term cultures; 3) yeast replicating inside phagocytes, unlike those outside or attached to the leukocyte surface, cannot take up uridine from the culture medium; and 4) supernatants obtained from cultures of leukocytes after incubating the cells in the presence or

TABLE 3

Adherence of PMNs from patients with acute gouty arthritis before and after colchicine treatment and of PMNs from normal individuals determined on two occasions 1 week apart

Normal Controls			Patients*		
Subjects	Adherence		Subjects	Adherence	
	Day 0	Day 7		Day 0	Day 7
	%	%		%	%
M. H.	67.4	52.0	J. B.	88.1	87.2
K. L.	48.5	62.3	R. B.	89.6	88.7
E. D.	69.7	67.6	N. C.	82.7	72.5
B. H.	62.5	58.9	T. F.	61.4	55.2
P. P.	84.2	75.2	A. D.	63.6	69.0
C. M.	66.2	55.2	J. S.	85.4	96.7
A. E.	47.8	61.6	N. D.	73.0	50.1
			A. D.	66.7	58.3
			A. J.	76.4	57.0
			J. O.	92.4	79.5
Mean ± S.E.	63.76 ± 4.78	61.83 ± 2.93	Mean ± S.E.	77.93 ± 3.59	71.42 ± 5.12

* Patients were treated with colchicine as described under "Methods." Blood samples were obtained just before the initiation of therapy (day 0) and then at 1 week after the initiation of therapy (day 7).

absence of yeast have no effect on the yeast uptake of [^{3}H] uridine. We modified this method to permit kinetic measurement and to improve reproducibility. The procedure that was finally adopted required only 5×10^4 neutrophils per determination. A single operator can perform 40 determinations in 3 hr.

Both the phagocytic capacities and the phagocytic rate of PMNs were found to vary widely among normal individuals. However, with any given individual, these values were found to be quite constant from 1 week to the next. Patients with acute gouty arthritis treated for 1 week with oral colchicine showed a statistically significant drop in both the phagocytic capacity and the phagocytic rate compared with the pretreatment values (tables 1 and 2). The reductions in phagocytic capacity appeared to be due to changes intrinsic to the cells because similar results were obtained when phagocytic capacities were determined in the presence of AB serum instead of fresh autologous serum.

The adherence of PMNs to capillary endothelium is one of the earliest events of inflammation. It is followed by diapedesis into the extravascular compartment, chemotaxis, phagocytosis and the release of a host of enzymes and mediators. Reductions of adherence of PMNs are therefore expected to decrease the accumulations of cells at the site of inflammation leading to a suppression of the inflammatory response. Standard anti-inflammatory agents, as well as gold and chloroquine, have been shown to cause suppressions of neutrophil adherence in the rat (Stecher and Chinea, 1978). In normal humans, oral administrations of aspirin (1.2 g) or prednisone (40 mg) produced prompt and statistically significant suppressions of neutrophil adherence (MacGregor, 1976). MacGregor (1976) has reported that the average value for the adherence of PMNs of five normal volunteers was reduced 29% after the administration of a single oral dose of colchicine (1.8 mg).

In the present sutdy, the adhesiveness of PMNs was found also to vary over a wide range among normal individuals. However, the values determined 1 week apart for a given individual were quite consistent. No change in PMN adhesiveness was observed in patients with acute gouty arthritis after 1 week of colchicine therapy (table 3). The discrepancy between the present findings and that reported by MacGregor (1976) may be due to the timing of blood sampling because the effect of colchicine on adherence may be transient. In the present study, the blood sample was collected at approximately 18 hr after the last oral dose of colchicine (1.8 mg), which was much later than that in the prior study (2–4 hr) (MacGregor, 1976). It may also be due to the presence of an inflammation-induced adherence augmenting factor in patients with gouty arthritis. This factor has been shown to be present in the plasma of patients with other acute inflammatory diseases including bacterial infection, systemic lupus erythematosus and acute myocardial infarctions, but not in that of normal subjects (Lentnek *et al.*, 1976). We conclude that in the setting of a clinical response of gouty arthritis to oral colchicine the phagocytic capacity and phagocytic rate of PMNs are reduced, but their adhesiveness is not. A study aimed to determine whether the observed reduction in phagocytic activity is due to the effect of colchicine or a reflection of the resolution of gouty inflammation is in progress.

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