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Development of Carrageenan Pleurisy in the Rat: Effects of Colchicine on Inhibition of Cell Mobilization (41229)

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Abstract. Colchicine produced three effects which modified the acute inflammatory response to carrageenan in the rat pleural cavity: (i) inhibition of neutrophil mobilization and concomitant exudate formation (3 hr); (ii) inhibition of monocyte mobilization (21 hr); and (iii) augmented exudate formation (3 and 21 hr). The 1st effect was related to the intraperitoneal dose of colchicine and occurred only at leukopenic dose levels. This effect could not be produced by intrapleural injection of nonleukopenic doses of colchicine. The second effect, on the other hand, was produced by intraperitoneal leukopenic doses and to a lesser extent by intrapleural administration of nonleukopenic doses of colchicine. Importantly, the normal biphasic exudative response to carrageenan developed fully in the absence of monocytes. The third effect, a dose-dependent augmentation of both exudative phases of carrageenan pleurisy, was produced by low, nonleukopenic, intrapleural doses of colchicine. The augmented exudate was sensitive to prostaglandin synthetase inhibitors but not to anti-inflammatory steroids. Neither neutrophils nor monocytes were responsible for the augmented exudate. Colchicine, injected into the rat hindlimb or pleural and peritoneal cavities did not elicit the mobilization of neutrophils or a pleural effusion. In addition, colchicine did not affect the magnitude, temporal development, or decay of the potent edematogenic action of serotonin in the rat hindlimb. Thus irritancy was not responsible for any of the effects of colchicine.

Colchicine was shown by Fruhman (1) to reduce the number of neutrophils mobilized in response to the intraperitoneal injection of bacterial lipopolysaccharide in the rat. More recently, colchicine was reported to reduce the number of neutrophils mobilized as a result of the intrapleural injection of each of three crystalline irritants (2). Interestingly, colchicine treatment produced a larger volume of pleural exudate at 4 hr compared to that seen in the control animals. Quantitative studies of exudate formation and cell mobilization in response to the algal polysaccharide carrageenan in the rat pleural cavity have shown that the time course of exudate formation is biphasic (first phase, 1–3 hr, second phase, 4–21 hr) (3, 4). Drug inhibition studies reinforced this biphasic concept (4, 5). Neutrophil mobilization took place in the first phase and monocyte mobilization occurred during the early part of the second phase (4–7 hr). Importantly, neutrophil mobilization was essentially complete before monocytes appeared in the pleural exudate. This temporal separation allowed us to study the effect of colchicine on the mobilization of each of these cell types. In some studies colchicine was injected intrapleurally in combination with a fixed dose of carrageenan. Small, intrapleural, doses of colchicine elicited local actions whereas large, intrapleural doses produced both local and systemic effects. Finally, dose-related actions of colchicine on the peripheral white blood cell count were determined. We now report the findings of these studies.

Materials and Methods. Male Sprague–Dawley rats weighing 160–180 g, purchased from ARS/Sprague–Dawley, Madison, Wisconsin, were used. The animals were caged individually and fasted 12 hr before dosing. Water was allowed *ad libitum*. Carrageenan (RE 8254, obtained from Marine Colloids, Inc., Rockville, Maine) was suspended in

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pyrogen-free water to a concentration of 0.40 or 0.06% (w/v) and constantly agitated on a magnetic stirrer. Each intrapleural injection (0.25 ml) was given between the third and fifth ribs on the right side of the mediastinum. The rats were sacrificed by CO_2 asphyxiation at various times after injection of carrageenan. Exudate volumes and the number of mobilized cells were then determined by methods described in a previous publication (3).

Each drug, including colchicine (Sigma No. C-9754), was suspended in a 0.5% medium viscosity sodium carboxymethylcellulose (CMC) gum (Hercules, Inc., Wilmington, Del.). The diluent was pyrogen-free water (Cutter Laboratories, Berkeley, Calif.). Drugs were administered orally, by gavage, or intraperitoneally in a volume of 1.00 ml/100 g body wt. In some studies colchicine was administered intrapleurally to each rat in a fixed volume of 0.25 ml of carrageenan (0.06%) or CMC (0.50%). In studies concerned with drug action on the first exudative phase of carrageenan pleurisy drugs were administered 30 or 60 min before carrageenan injection and the exudate was harvested 3 hr after injection of the irritant. In studies concerned with drug action on the second exudative phase of carrageenan pleurisy drugs were administered after the development of the first phase (4 hr after intrapleural injection of carrageenan unless otherwise specified) and the exudate was harvested 21 hr after irritant injection.

Histology. For histological study of the pleural inflammatory response in carrageenan-injected rats the exudate was harvested and the resident and mobilized cells were washed from the cavity with 0.10% disodium ededate in 0.90% pyrogen-free saline. A portion of the thorax, including the sternum, the right (injected) side of the ribcage, and the diaphragmatic tissue were then removed intact and fixed for a minimum of 24 hr in 10% neutral phosphate-buffered formalin. A 2-cm-long and 1-cm-wide strip of tissue was cut approximately 0.5 cm to the right of and parallel to the sternum which included a 1-cm section of diaphragm attached perpendicularly to an equal area of ribcage. The tissue was then embedded in paraffin such that the 90° angle between the ribcage and the diaphragm was preserved. Saggital sections of the embedded tissues were made, mounted on glass slides, and stained with hematoxylin and eosin. Each tissue was scored on an arbitrary scale of 0 to +4 for the presence and number of perivascular neutrophils (in diaphragm muscle), subpleural neutrophils, and subpleural edema. The edema was characterized by enlarged spaces in subpleural connective tissue in the region overlying the diaphragm muscle that were filled with a homogeneous, faintly staining eosinophilic material, representing edema protein. The observer was unaware of the treatment of each animal. To provide consistency from tissue to tissue the observer scored each section observing only the diaphragm beginning in the region of the angulation where the diaphragm reflects away from the ribcage. The tissue of the ribcage was not scored.

Results. *Dose-dependent actions of intraperitoneal and intrapleural colchicine on carrageenan pleurisy.* Intraperitoneal administration of colchicine reduced the number of neutrophils mobilized and the volume of exudate formed in response to carrageenan at 3 hr. The magnitude of these reductions were dose related (Table 1). The doses and SEM of colchicine required to reduce the number of mobilized neutrophils and the exudate volume 50% were 0.97 ± 0.070 and 1.5 ± 0.67 mg/kg, respectively, as calculated by regression of log dose vs effect.

The intrapleural injection of a wide range of doses of colchicine (0.02–20.0 μg/rat) in combination with a fixed amount of carrageenan produced a dose-dependent increase in the volume of exudate harvested at 3 hr (Table II). The number of neutrophils mobilized was not reduced in this dose range. At a higher dose, 200 μg/rat, the number of neutrophils mobilized and the volume of exudate formed was significantly decreased (Table II). The 200 μg/rat dose is equivalent to a 1.1 mg/kg dose in our rats which weighed, on average, 180 g.

In another study various doses of colchicine were injected intraperitoneally 4 hr after carrageenan and the exudate was har-

TABLE I. DOSE-DEPENDENT ACTIONS OF COLCHICINE ON FIRST PHASE OF CARRAGEENAN PLEURISY

Drug	Dose (mg/kg, ip)	Three-hour exudate volume (ml ± SEM)	Percentage change in 3-hr exudate volume	Neutrophils mobilized at 3 hr × 10^6 cells ± SEM)	Percentage change in No. of Neutrophils mobilized at 3 hr
Solvent control	—	0.51 ± 0.056	—	40.6 ± 4.29	—
Colchicine	0.03	0.45 ± 0.052	−11	44.5 ± 2.66	+9
	0.12	0.47 ± 0.057	−8	40.1 ± 2.88	−1
	0.50	0.39 ± 0.057	−24	35.7 ± 3.51	−12
	2.00	0.22 ± 0.026″	−57	3.2 ± 2.10″	−92

Note. Colchicine was injected intraperitoneally 30 min before 150 μg of carrageenan. The pleural exudate was harvested 3 hr after the intrapleural injection of carrageenan ($N = 6$).

″ Statistically significant difference from control ($P < 0.05$, by Student's t test).

vested 21 hr after the carrageenan injection. This regimen allowed the study of the effect of colchicine on monocyte mobilization and the second-phase pleural effusion since at 4 hr all of the first phase volume has been produced, neutrophil mobilization is essentially complete, and monocyte mobilization is just starting. Colchicine produced a dose-dependent increase in the volume of the second-phase exudate produced by carrageenan (Table III). This augmentation was maximal, 65% at the 1.5 mg/kg level ($P < 0.01$). When the dose of colchicine was increased to 2.0 mg/kg the magnitude of the increase was reduced. A dose-dependent reduction in the number of monocytes mobilized was elicited by intraperitoneal administration of colchicine (Table III). The dose of colchicine calculated to reduce the number of monocytes mobilized by 50% was 0.51 ± 0.215 mg/kg. Inhibition of monocyte mobilization was essentially complete (94 and 99%) at dose levels which both increased the volume of the second-phase exudate (1.5 mg/kg) and reduced the augmentation (2.0 mg/kg).

The intrapleural injection of colchicine 4 hr after a fixed quantity of carrageenan affected the volume of pleural effusion and the number of monocytes mobilized in the 21-hr (second-phase) exudate (Table IV). Dose levels of 2.0 and 20.0 μg/rat aug-

TABLE II. LOCAL ACTIONS OF COLCHICINE ON 3-hr CARRAGEENAN PLEURAL EXUDATE

		Three-hour carrageenan pleurisy			
Irritant + drug	Dose (μg/rat, ipl)	Edema volume (ml ± SEM)	− % inhibition, + % stimulation	Neutrophils mobilized (× 10^6 ± SEM)	− % inhibition, + % stimulation
Carrageenan no drug	—	0.73 ± 0.098	—	47.4 ± 5.11	—
Carrageenan + colchicine	0.02	0.92 ± 0.052 (NS)	+26	48.0 ± 4.57 (NS)	+1
	0.20	0.97 ± 0.126 (NS)	+33	50.1 ± 4.06 (NS)	+6
	2.00	1.08 ± 0.055 ($P < 0.02$)	+48	54.3 ± 5.16 (NS)	+15
	20.00	1.42 ± 0.108 ($P = 0.0001$)	+95	42.8 ± 3.83 (NS)	−10
	200.00	0.45 ± 0.072 ($P < 0.05$)	−38	7.5 ± 1.73 ($P < 0.0001$)	−84

Note. Each rat received an intrapleural injection of 150 μg of carrageenan with or without colchicine ($N = 6$) and the exudate was harvested 3 hr later. NS = not significant ($P > 0.05$, by Student's t test).

TABLE III. DOSE-DEPENDENT ACTIONS OF COLCHICINE ON SECOND PHASE OF CARRAGEENAN PLEURAL EXUDATE

Drug	Dose (mg/kg, ip)	Volume of 21-hr exudate (ml, mean ± SEM)	Percentage change in 21-hr exudate volume	Monocytes in 21-hr exudate × 10^6 (mean ± SEM)	Percentage change in 21-hr monocyte count
Solvent, control	—	1.25 ± 0.159	—	40.2 ± 3.31	—
Colchicine	0.12	1.42 ± 0.206 (NS)	+14	39.2 ± 6.07 (NS)	−2
	0.50	1.50 ± 0.215 (NS)	+20	24.4 ± 4.48 ($P < 0.02$)	−39
	1.50	2.05 ± 0.175 ($P < 0.01$)	+65	2.5 ± 1.39 ($P < 0.0001$)	−94
	2.00	1.49 ± 0.206 (NS)	+19	0.2 ± 1.65 ($P < 0.0001$)	−99

Note. In this experiment 1000 μg of carrageenan was injected intrapleurally ($N = 8$). Colchicine was injected intraperitoneally 4 hr later. Exudates were harvested 21 hr after injection of carrageenan. NS = not significant ($P > 0.05$, by Student's t test).

mented exudate volume 54 and 91%, respectively ($P < 0.05$ for each). When the intrapleural dose was increased to 200 μg/rat the augmented volume was reduced. Intrapleural administration of colchicine produced a dose-dependent reduction in the number of monocytes mobilized (Table IV). This action was biphasic, first consisting of a gradual reduction between the doses of 0.2 and 20 μg/rat and then followed by a greater reduction between 20 and 200 μg/rat. This action of colchicine was statistically significant at the 20 μg/rat dose level ($P < 0.05$). The dose of colchicine calculated to reduce the number of monocytes mobilized by 50% was 36 μg/rat which in rats weighing an average of 180 g represents a dose of approximately 0.20 mg/kg.

The temporal development of exudate volume and neutrophil mobilization produced by carrageenan (150 μg) with and without colchicine (2.5 μg) over the first 3 hr is shown in Fig. 1. Twenty minutes after injection no exudate was recovered from animals receiving carrageenan alone or in combination with colchicine. At 60 min a small volume of exudate was harvested from both groups. The volume obtained from the group which received the combination of drugs was larger, statistically, than that harvested from the group receiving only carrageenan ($P < 0.02$). A similar effect was observed at 120 min although the exudate volume of each group increased relative to the 60-min volume. At 180 min after injection, animals that received the irritant with colchicine produced a large volume of pleural effusion, 1.17 ml, approxi-

TABLE IV. LOCAL DOSE-DEPENDENT ACTIONS OF COLCHICINE ON SECOND PHASE OF CARRAGEENAN PLEURISY

Drug	Dose (μg/rat, ipl)	Volume of 21-hr exudate (ml, mean ± SEM)	Percentage change in 21-hr exudate volume	Monocytes in 21-hr exudate × 10^6 (mean ± SEM)	Percentage change in 21-hr monocyte count
Solvent, control	—	1.38 ± 0.217	—	40.7 ± 3.24	—
Colchicine	0.2	1.39 ± 0.235 (NS)	+1	36.9 ± 3.92 (NS)	−9
	2.0	2.13 ± 0.178 ($P < 0.05$)	+54	31.6 ± 3.06 (NS)	−22
	20.0	2.64 ± 0.238 ($P < 0.01$)	+91	27.4 ± 4.74 ($P < 0.05$)	−33
	200.0	1.73 ± 0.184 (NS)	+25	0.1 ± 0.10 ($P < 0.01$)	−100

Note. In this experiment 1000 μg of carrageenan was injected intrapleurally ($N = 7$). Colchicine was injected intrapleurally 4.0 hr later. Exudates were harvested 21 hr after injection of carrageenan. NS = not significant ($P > 0.05$, by Student's t test).

mately twofold in excess of the exudate volume of 0.54 ml produced by carrageenan alone. The number of neutrophils mobilized by each treatment was the same at 20, 60, 120, and 180 min (Fig. 1).

Drug actions. The average exudate volume harvested 3 hr after a 1000-μg dose of carrageenan is 0.8 ml. This volume is termed the 1st phase (Table V) and is inhibited in a dose-related manner by both nonsteroidal and steroidal anti-inflammatory drugs. Colchicine (1.5 mg/kg, ip) administered prior to carrageenan does not change the magnitude of the first-phase volume. However, colchicine pretreatment changed the sensitivity of the first phase to drugs (Table V). The first-phase sensitivity to PG synthetase inhibitors increased or remained the same in the colchicine-treated animals whereas the steroids lost potency. Colchicine administered 4 hr after carrageenan (after the first phase is developed) produces a demonstrable increase in the second-phase (21 hr) exudate volume from 1.5 to 2.2 ml, on average. The drug sensitivity of the second phase is also altered by colchicine. For example, aspirin and indomethacin were inactive against the development of the second-phase exudate volume produced by carrageenan alone whereas the steroids were very potent inhibitors of this volume (Table V). In colchicine-injected animals aspirin and indomethacin produced a limited but significant degree of inhibition ($P < 0.05$ and $P < 0.02$, respectively) of the second-phase carrageenan exudate. The potency of the steroids against the second-phase edema in colchicine-treated animals was decreased sixfold relative to their potency in untreated animals. Importantly, it was noted that upper dose levels of steroids could completely block the second-phase exudate in normal animals. However, in colchicine-treated rats an upper limit of only 60% inhibition was attainable.

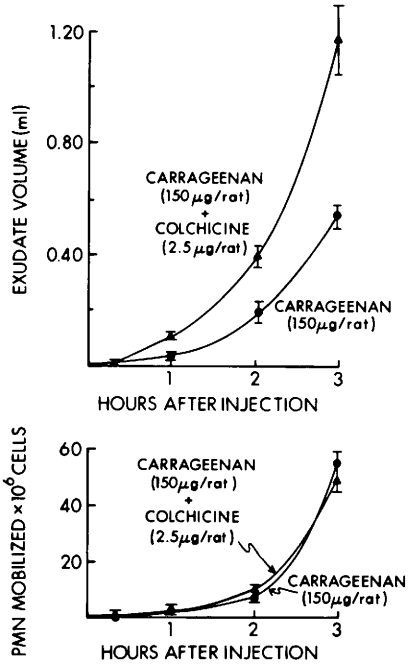

FIG. 1. Pleural inflammation produced in the rat by intrapleural injection of carrageenan (150 μg) alone or in combination with colchicine (2.5 μg).

Irritancy and the effects of colchicine on the peripheral WBC. The intraperitoneal injection of 1.5 mg/kg of colchicine did not produce an inflammatory response in the peritoneal or pleural cavity at 20 and 180 min (results not shown). When colchicine was injected into the rat pleural cavity in the range of 0.04–0.64 mg/rat, no pleural effusion or cell mobilization was detected at 20, 60, or 180 min. Importantly, the number of free resident cells in the pleural cavity was not changed by the intrapleural injection of colchicine. Subplantar injection of the same doses of colchicine also failed to produce a measureable effusion. In this experiment the subplantar injection of 1.0 μg of serotonin elicited average edema volumes of 0.056 ± 0.042, 0.59 ± 0.039, and 0.43 ± 0.032 ml at 20, 60, and 180 min, respectively ($N = 5$). Colchicine, 1.5 mg/kg, intraperitoneally, or 0.020 mg/rat, intrapleurally, did not change the magnitude or temporal development and decay of the rat hindlimb edema produced by serotonin. Thus the action of colchicine is specific for an inflammatory (carrageenan) but not an edemagenic (serotonin) reaction. An inflammatory reaction is defined by edema which is dependent on the mobilization of phagocytic cells for its development whereas an edemagenic reaction is defined

TABLE V. DRUG ACTION IN THE CARRAGEENAN PLEURISY ASSAY WITH AND WITHOUT COLCHICINE PRETREATMENT

Drug	Carrageenan pleurisy assay[a] (oral ED_{50}[b], mg/kg)			
	First-phase exudate volume		Second-phase exudate volume	
	No pretreatment	Colchicine (1.5 mg/kg, ip)	No pretreatment	Colchicine (1.5 mg/kg, ip)
Aspirin	41 ± 3.6	23 ± 2.1	Inactive at 150 mg/kg	>150 mg/kg
Indomethacin	1.8 ± 0.22	1.6 ± 0.30	Inactive at 3 mg/kg	>3 mg/kg
Corticosterone	30 ± 6.3	>120 mg/kg	12 ± 1.4	72 ± 27.9
Prednisolone	7.7 ± 1.77	>24 mg/kg	0.8 ± 0.03	4.7 ± 2.20

[a] Each rat received an intrapleural injection of 1000 μg of carrageenan in 0.25 ml of pyrogen-free water. For studies of the first phase drugs were administered po and colchicine or the solvent, carboxymethylcellulose (0.5%) injected ip, 60 and 30 min, respectively, before the intrapleural injection of carrageenan ($N = 5$). For studies of the second phase drugs were administered po and colchicine or the solvent injected ip, 3 hr and 45 min and 4 hr and 15 min, respectively, after the intrapleural injection of carrageenan ($N = 6$). Exudates were harvested 3 hr (first phase) and 21 hr (second phase) after carrageenan injection.

[b] ED_{50} is the dose which reduced the average exudate volume of a solvent-injected control by 50% as estimated by regression of log dose vs effect.

by edema which is independent of the mobilization of phagocytic cells.

The time course of the effects of colchicine, 1.5 mg/kg, intraperitoneally, on the peripheral white blood cell count revealed that 1 and 3 hr after injection the number of circulating neutrophils and lymphocytes was reduced. In a subsequent study the neutropenic action of colchicine 1 hr after intraperitoneal injection was found to be dose-dependent (Table VI). The dose needed to produce a 50% reduction in circulating neutrophils was calculated to be 1.2 ± 0.10 mg/kg. When various doses of colchicine were injected intrapleurally with a fixed quantity of carrageenan (150 μg/rat) and the total and differential white blood cell counts taken on blood samples drawn at 1 hr a dose-dependent decrease in the number of circulating neutrophils was produced. The dose of colchicine calculated to reduce the peripheral neutrophil count by 50% was 0.49 ± 39.9 μg/rat which in animals weighing 180 g averaged to a dose of 0.27 mg/kg. The intrapleural injection of carrageenan alone (150 μg/rat) did not reduce the peripheral neutrophil count at 1 hr (results not shown).

TABLE VI. DOSE-DEPENDENT ACTIONS OF COLCHICINE ON PERIPHERAL WBC OF THE RAT[a]

Drug	Dose (mg/kg, ip)	Peripheral WBC × 10^6 cells/ml (mean ± SEM)	Percentage change	Peripheral neutrophils × 10^6 cells/ml (mean ± SEM)	Percentage change	Peripheral lymphocytes × 10^6 cells/ml (mean ± SEM)	Percentage change
Solvent, control	—	19.9 ± 1.3	—	4.1 ± 0.45	—	15.3 ± 0.98	—
Colchicine	0.12	18.0 ± 1.42 (NS)[b]	−9	3.9 ± 0.49 (NS)	−5	13.8 ± 1.10 (NS)	−10
	0.50	19.0 ± 1.89 (NS)	−4	3.8 ± 0.44 (NS)	−7	15.0 ± 1.59 (NS)	−2
	1.5	13.5 ± 0.97 ($P < 0.01$)	−32	1.7 ± 0.28 ($P < 0.01$)	−58	11.7 ± 0.78 ($P < 0.05$)	−23
	2.0	9.7 ± 0.64 ($P < 0.001$)	−51	0.9 ± 0.05 ($P < 0.0001$)	−78	8.6 ± 0.58 ($P < 0.0001$)	−49

[a] In this experiment peripheral WBC counts of 30 rats were determined. The animals were then placed into five groups of 6 rats such that the average WBC count of each group was statistically equivalent to that of all other groups at the beginning of the experiment. Colchicine or the solvent, carboxymethylcellulose (0.5%), was injected ip and peripheral WBC counts were redetermined 1 hr later.

[b] NS = not significant ($P > 0.05$, by Student's t test).

Histological observations of diaphragmatic tissue. The effects of colchicine (1.5 mg/kg, intraperitoneally) on the histology of diaphragmatic tissue 3 hr after the intrapleural injection of 150 μg of carrageenan are shown in Table VII. The mean scores for the presence of neutrophils in the subpleural and perivascular tissue were reduced by colchicine ($P < 0.05$ and $P < 0.02$, respectively). In contrast, colchicine increased the degree of subpleural edema relative to the untreated group ($P < 0.02$). Differences in mean scores are not proportional to absolute changes in magnitude.

Discussion. Colchicine produced three effects which modulated the development of carrageenan pleurisy: (i) inhibition of neutrophil mobilization and concomitant exudate formation; (ii) inhibition of monocyte mobilization; and (iii) augmented exudate formation. For clarity these effects will be discussed independently insofar as possible.

Inhibition of neutrophil mobilization. After either intraperitoneal or intrapleural injection of colchicine the magnitude of inhibition of neutrophil mobilization was related to dose (Tables I and II). The dose calculated to reduce the number of neutrophils mobilized by 50% at 3 hr by either route of administration was of the same order of magnitude (0.96 ± 0.070 mg/kg, ip, Table I: 70 ± 10.0 μg/rat or 0.39 mg/kg, intrapleurally, in rats weighing 180 g on average, Table II). The closeness of these ED_{50} doses suggests that local action was not obtained. In general, local ED_{50}'s are 1/20th or less of intraperitoneal (systemic) ED_{50}'s in the rat. Importantly, to obtain inhibition of neutrophil mobilization by either route of administration it was necessary to inject dose levels which produced a peripheral neutropenia. A similar correlation was obtained when colchicine was used to suppress urate crystal-induced canine joint inflammation. That is, only neutropenic doses were effective (6). Colchicine has been reported to reduce the number of neutrophils mobilized in response to the intraperitoneal injection of endotoxin at a dose level which elicits a frank but transient neutropenia in the rat (1). Since the mobilized neutrophils are derived from the peripheral circulation (7, 8), a transient neutropenia which transpires during hours 1–3, when the first exudative phase is developing could account for the reduced numbers of neutrophils harvested at 3 hr in the current studies and at 5 hr in response to intraperitoneal endotoxin (1). It is unlikely that this action of colchicine is mediated by the reported antimitotic actions of this drug since it occurs rather rapidly although it may be related to its effects on sol–gel interactions (11).

Inhibition of monocyte mobilization. This effect was dose dependent after intraperitoneal or intrapleural injection of colchicine (Tables III and IV). The intrapleural route elicited a local action which had a flat dose response in the 0.2–20.0 μg/rat range. An intrapleural dose of only 2 μg/rat (approximately 0.01 mg/kg) produced a measureable but insignificant reduction in monocyte mobilization ($P > 0.05$, Table IV). It is unlikely that this low dose level is associated with a reduced number of mono-

TABLE VII. EFFECT OF COLCHICINE ON HISTOLOGY OF RAT DIAPHRAGMS 3 hr AFTER INTRAPLEURAL INJECTION OF CARRAGEENAN

Drug	Dose (mg/kg, ip)	Mean histology score[a] ± SEM of rat diaphragms 3 hr after intrapleural injection of carrageenan (150 μg)		
		Subpleural neutrophils	Perivascular neutrophils	Subpleural edema
Solvent control	—	1.7 ± 0.36	1.6 ± 0.27	1.1 ± 0.20
Colchicine	1.5	0.7 ± 0.17 ($P < 0.05$)	0.7 ± 0.11 ($P < 0.02$)	2.1 ± 0.31 ($P < 0.02$)

[a] Histology was rated on an arbitrary scale of 0 (no neutrophils or edema) to 4 (many neutrophils and severe edema). Differences in mean scores are not proportional to absolute changes in magnitude. There were six animals in each group.

cytes in the peripheral circulation since a 10-fold higher dose failed to affect the peripheral white blood cell count (see end of Results). In the 20–200 μg/rat dose range, however, a sharp reduction in monocyte mobilization took place indicating that a new component had been added to the gradual local action. The 200 μg/rat dose elicited a peripheral leukocytopenia which could account for the sharp reduction in monocyte mobilization. The mechanism responsible for the local reduction in monocytes is unknown. Importantly, the second-phase volume as well as the augmented volume developed fully in spite of the dose-related decrease in the number of monocytes mobilized (Tables II and IV) indicating that these edemas did not depend on monocytes for their development. Since the 1st exudative phase of carrageenan pleurisy is fully developed at 3 hr, before monocytes are mobilized (3) it can be surmised that the biphasic pleural effusion as well as the augmented effusion do not depend on monocytes for their production.

Augmentation of carrageenan pleural effusion. Intraperitoneal or intrapleural injection of colchicine augmented carrageenan exudate formation. The magnitude of the augmentation was dependent on the dose of colchicine (Tables II, III, and IV). The inhibitory activity of the anti-inflammatory steroids against the first-phase carrageenan exudate volume was reduced to the extent that ED_{50} values could not be obtained in colchicine-treated animals (Table V). This decrease in sensitivity indicates that colchicine had added a component to the first-phase exudate of carrageenan which was insensitive to anti-inflammatory steroids. The sensitivity of the first-phase carrageenan edema to the prostaglandin synthetase inhibitors, aspirin and indomethacin, was enhanced or remained unchanged after treatment with colchicine suggesting that a component added by colchicine, like the normal component (9) was dependent on prostaglandin biosynthesis for its inception and development.

In normal animals the second phase of carrageenan exudate formation is highly sensitive to anti-inflammatory steroids (Table V, note lower ED_{50}'s of steroids against second phase). In colchicine-treated carrageenan injected animals this high sensitivity to steroids was lost as a consequence of the insensitive augmented volume. The potency of the steroids thus decreased sixfold. Contrarily, the sensitivity of the second-phase exudate to prostaglandin synthetase inhibitors increased after colchicine treatment. That is, the synthetase inhibitors were devoid of activity against the normal second-phase volume but significant inhibition of the synthetase-sensitive, augmented second-phase volume was attained (Table V). These findings suggest that the second-phase edema harvested from colchicine-treated animals consisted of two components, the normal exudate elicited by carrageenan which is very sensitive to steroids but insensitive to synthetase inhibitors and the colchicine-induced component which has the inverse drug sensitivity. In retrospect, it appears that colchicine added a new component of pleural effusion to each phase rather than enhance the biphasic exudative response of carrageenan. This new component is sensitive to prostaglandin synthetase inhibitors and insensitive to anti-inflammatory steroids. It does not result from any irritancy of colchicine itself since none was detected in the animal models we used. In addition, augmented exudate formation can not be explained by a nonspecific action of colchicine on the local blood vessels (2) since the intraperitoneal or intrapleural injection of this antimitotic agent did not modify the development or decay of the potent, noninflammatory, edematogenic activity of serotonin in the rat hindlimb. The augmented edema may be explained by the ability of colchicine to increase prostaglandin biosynthesis of the tissue of the pleura since it has been shown to stimulate formation of prostaglandin E in human rheumatoid synovial organ cultures (10).

In summary, inhibition of neutrophil and monocyte mobilization by colchicine was used to study some of the mechanisms responsible for the biphasic exudative development of carrageenan pleurisy. Our results suggest that neutrophils are in some

way responsible for the first exudative phase whereas monocytes are not responsible for any exudate formation.

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