Some Quantitative Temporal Characteristics of Carrageenin-Induced Pleurisy in the Rat (37397)

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(Introduced by C. H. Ellis)
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Studies by Fruhman (1, 2) reveal the mobilization of large numbers of neutrophils in response to an intraperitoneal injection of endotoxin in the rat and describe a quantitative technique for recovering these cells. The intrapleural injection of kaolin also produces an inflammatory response in which it is possible to recover quantitatively and identify the inflammatory cells that are mobilized. Steroidal and nonsteroidal anti-inflammatory drugs inhibit the pleural mobilization of neutrophils elicited by kaolin (3). Subplantar injection of the algal polysaccharide, carrageenin, produces edema and inflammation in the subcutaneous tissue of the rat hindlimb (4, 5). The intrapleural injection of this irritant has recently been shown to produce an exudate (6). We now report the quantitative temporal characteristics of the accumulation of inflammatory cells and exudate following the intrapleural injection of carrageenin in normal and aspirin-treated rats.

Materials and Methods. Male Sprague–Dawley rats, 180 ± 20 g obtained from ARS/Sprague–Dawley, Madison, Wisconsin were used. The animals were caged individually and food was withheld the night before an experiment. Water was given *ad libitum*. The carrageenin (0.20%) was made up in pyrogen-free water 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 animals were restained but not anesthetized. At predetermined intervals, after injection of the irritant, the animals were sacrificed with chloroform and the skin and pectoral muscles retracted leaving the ribcage exposed. A longitudinal incision was made between the third and fifth ribs on each side of the mediastinum. A tuberculin syringe with a 16-gauge intubation needle affixed was used to aspirate the fluid from the right and left aspects of the thoracic cavity. The exudate was transferred to a 15 ml conical centrifuge tube and the total volume determined by drawing the fluid back into the calibrated tuberculin syringe. Both sides of the thoracic cavity were flushed with a volume of 0.10% EDTA in saline which when added to the exudate totaled 5.0 ml. A 50-lambda aliquot was used to determine the total leukocyte count. The remaining fluid was centrifuged for four minutes at 900 rpm and the cells resuspended in 0.15 ml of horse serum. Smears were made, allowed to air dry, and stained with buffered Wright's stain. Aspirin, triprolidine, and cyproheptadine were suspended in a 0.5% medium viscosity carboxymethylcellulose gum (Hercules, Inc., Wilmington, Delaware) and administered by gavage 30 min prior to the intrapleural injection of carrageenin. The doses were 90, 1, and 5 mg/kg, respectively. All control animals received 1.00 ml/100 g body wt of the suspending agent. There were 5 rats in each group.

Results. The time course of exudate formation which resulted from the intrapleural injection of 500 μg of carrageenin was sigmoid over the first 5 hr (Fig. 1). There was a one hour delay before any fluid above control level could be aspirated. A sharp increase in exudate formation occurred between the 1st and 3rd hr. More than 70% of the maximum exudate volume of 1.08 ml was formed in this 2-hr interval. The 3-hr volume was equal, statistically, to the 5-hr volume. A second

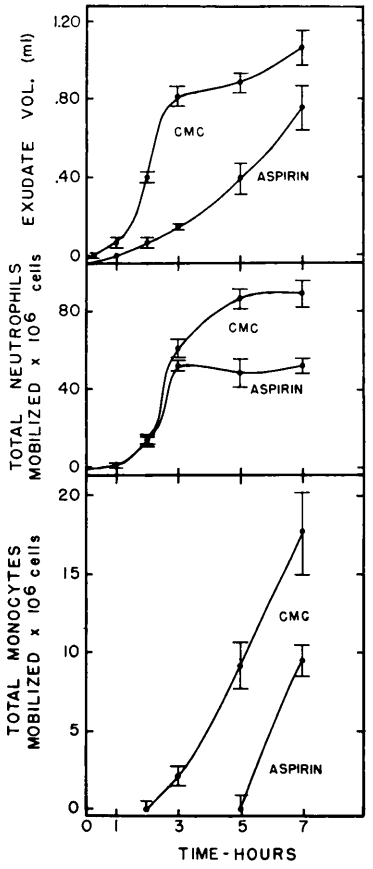

FIG. 1. Time course of exudate, neutrophil and monocyte accumulation resulting from the intrapleural injection of 500 μg of carrageenin in carboxymethylcellulose (CMC) and aspirin-treated animals. Aspirin (90 mg/kg) or CMC, the solvent for the aspirin (0.5%), was given orally thirty minutes before the irritant. Mean values of two experiments each containing 5 rats/group are plotted. The vertical bars represent the standard error of the mean.

period of accelerated exudate formation appears to be starting at 5 hr. Preliminary experiments at 16 and 24 hr confirm this observation. Pretreatment with aspirin had a pronounced but transient effect on exudate formation (Fig. 1). At 3-hr exudate formation was inhibited 83% by aspirin. After the third hr, exudate formation increased in the aspirin-treated animals, and by the 7th hr the inhibition was only 31%. The exudate–time curve resulting from the intrapleural injection of 100 μg of carrageenin was also sigmoid (Fig. 2). Both the 100 and 500 μg pleural exudate–time curves were sigmoid over the first five hrs which indicated the occurrence of only one period or phase of accelerated edema formation (Fig. 2). The hindlimb injection of 500 μg of carrageenin produced a curve with 2 sigmoid sections (Fig. 2). More than 75% of the first phase of the hindlimb edema is produced within 20 min of the injection of the irritant.

The time course of neutrophil mobilization resulting from the intrapleural injection of carrageenin was sigmoid, and increased sharply between the 1st and 3rd hr (Fig. 1). Approximately 56 million neutrophils were mobilized during this 2-hr interval. Neutrophil mobilization decelerated and ceased between the 3rd and 7th hr. Aspirin inhibited the number of neutrophils mobilized at 3 hr by 26% and blocked all subsequent mobilization through the 7th hr.

There was a 2 hr delay before any monocytes were mobilized in response to the intrapleural injection of carrageenin (Fig. 1). After this delay, the rate of monocyte mobilization was nearly linear with respect to time through the 7th hr. Aspirin completely inhibited monocyte mobilization for the 1st 5 hr. From the 5th through the 7th hr the rate of monocyte mobilization was the same in control and aspirin-treated rats. The antihistaminic triprolidine and the antiserotonin

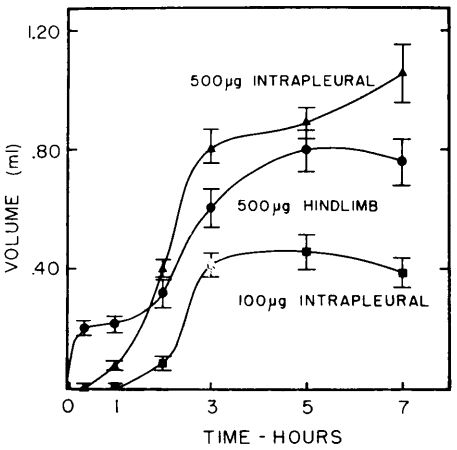

FIG. 2. Edema volume produced in response to the intrapleural and subplantar injection of carrageenin, 10 rats in each group. The vertical bars represent the standard error of the mean.

agent, cyproheptadine did not affect pleural exudate formation or the mobilization of neutrophils and monocytes.

Discussion. In response to the subplantar and intrapleural injection of 500 μg of carrageenin different edema–time curves were obtained in the first 5 hr. The pleural curve was monophasic and the hindlimb curve biphasic (Fig. 2). There was no indication of a third period of fluid accumulation in the hindlimb edema–time curve during the 7-hr period as suggested by DiRosa *et al.* (6). The first phase of the hindlimb edema–time curve developed in 1 hr and was not inhibited by aspirin at 100 mg/kg (7). This rapidly developing first phase did not have a counterpart in the pleural exudate–time curve. The monophasic accumulation of intrapleural fluid corresponded in both magnitude and temporal development to the second phase of the hindlimb edema. Additional evidence for a similar etiology of these edemas is their sensitivity to drugs. Both edemas are strongly inhibited by relatively low oral doses of aspirin and both are insensitive to the antihistaminic triprolidine and the antiserotonin agent cyproheptadine.

Histological examination of tissue sections of the rat hindlimb taken 20, 60, and 180 min after the subplantar injection of carrageenin revealed the temporal characteristics of neutrophil mobilization (8). Interestingly, these qualitative histological observations can readily be correlated with the neutrophil mobilization which takes place during the development of carrageenin pleurisy. Twenty minutes after the subplantar injection of carrageenin the mobilized neutrophils were localized in the capillaries near the injection site. None of these cells had traversed the vessel wall. There were no neutrophils in the 20-min pleural exudate of carrageenin-injected animals. At 60 min a small portion of the neutrophils mobilized in the hindlimb and migrated through and beyond the capillary walls and were free in the subcutaneous tissue while the remainder were either perivascular, intramural, or intravascular. A small but significant number of neutrophils were found in the 60 min pleural exudate. The hindlimb tissue sections taken 180 min after injection of carrageenin revealed the vast majority of the mobilized neutrophils to be free in the subcutaneous tissue. Few neutrophils remained in the capillaries or were traversing the vessel walls. The 180-min pleural exudate contained 60 million neutrophils, a 56 million increase over the 60-min exudate. These results suggest that the appearance of neutrophils free in the subcutaneous tissue or pleural cavity in response to the irritant, carrageenin, are equivalent. The advantage of studying pleural inflammation is that both the identity and the absolute number of inflammatory cells mobilized can readily be determined. In the interval between the first and third hour, 56 million neutrophils were mobilized in response to the intrapleural injection of 500 micrograms of carrageenin. This is a larger and more rapid mobilization than that produced by 0.1 μg of endotoxin injected into the peritoneal cavity (2).

The appearance of large numbers of neutrophils free in the subcutaneous tissue was temporally related to the development of the large second phase hindlimb edema. Similarly, the appearance of large numbers of neutrophils in the pleural cavity in the 1–3 hr period corresponded to the interval of maximum exudate formation. The temporal characteristics of pleural neutrophil mobilization and exudate formation are both sigmoid, monophasic, and increase sharply between the 1st and 3rd hr. This parallelism suggests that these phenomena are related and may also be construed as evidence in support of the concept that lysosomal enzymes released from neutrophils are responsible for the acute edematous reaction to some irritants (9).

During the acute stage of carrageenin pleurisy, monocyte mobilization began 2 hr after appreciable numbers of neutrophils were mobilized. This sequence of cell accumulations is similar to those observed microscopically during the development of many inflammatory responses in humans (10). The temporal characteristics of monocyte mobilization and exudate formation are different. There does not appear to be any interdependence of these parameters. Half of the maximum exudate volume is produced before any monocytes are mobilized and, in addition, most of

these cells are mobilized between the third and seventh hour when only a small portion of the exudate is produced. It is interesting to note that aspirin completely curtailed the mobilization of monocytes and neutrophils between the third and fifth hr when the effect of this drug on exudate formation was waning.

Summary. The intrapleural injection of 500 μg of carrageenin produces an acute inflammatory reaction in the rat in which it is possible to recover quantitatively the inflammatory cells that are mobilized and the exudate. The time courses of exudate formation and neutrophil mobilization strongly suggest that these phenomena are related. Both curves are sigmoid, and increase sharply between the first and third hours. Aspirin at 90 mg/kg, p.o., produced 83% inhibition of exudate formation at three hours but by the seventh hour the inhibition was only 31%. Neutrophil mobilization was only slightly inhibited by aspirin at three hours. However, the neutrophil mobilization which occurred between the third and seventh hours was completely blocked by aspirin. Monocyte mobilization started two hours after injection of the carrageenin and continued at a fixed rate through the seventh hour. There was no mobilization of monocytes in the aspirin-treated animals for the first five hours. Thereafter the rate of mobilization of these cells was similar in control and aspirin-treated animals.

Three lines of evidence suggest that the single period of edema formation produced by the intrapleural injection of carrageenin in the first 5 hr is analogous to the second phase of the biphasic rat hindlimb reaction to this irritant. The major portion of these edemas is formed 1–3 hr after injection of carrageenin. Temporally associated with the development of both edemas is the mobilization of large numbers of neutrophils and the appearance of these cells free in the inflamed tissue. Finally, both edemas are strongly inhibited by relatively low doses of aspirin and both are insensitive to the antihistaminic triprolidine and the antiserotonin agent cyproheptadine.

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1. Fruhman, G. J., Proc. Soc. Exp. Biol. Med. **102**, 423 (1959).

2. Fruhman, G. J., Blood. **16**, 1753 (1960).

3. Vinegar, R., Truax, J. F., and Selph, J. L., 5th Int. Cong. Pharmacol., Abstracts of Volunteer Papers, 1972.

4. Winter, C. A., Risley, E. A., and Nuss, G. W., Proc. Soc. Exp. Biol. Med. **111**, 544 (1962).

5. Van Arman, C. G., Begany, A. J., Miller, L. M., and Pless, H. H., J. Pharmacol. Exp. Ther. **150**, 328 (1965).

6. DiRosa, M., Giroud, J. P., and Willoughby, D. A., J. Pathol. **104**, 15 (1971).

7. Vinegar, R., Schreiber, W., and Hugo, R., J. Pharmacol. Exp. Ther. **166**, 96 (1969).

8. Vinegar, R., Macklin, A. W., Truax, J. F., and Selph, J. L., Pharmacologist **13**, 284 (1971).

9. Weissmann, G., and Thomas, L., *in* "Bacterial Endotoxins" (M. Landy and W. Braun, eds.), p. 602. Rutgers University Press, New Jersey (1964).

10. Wilhelm, D. L., *in* "Pathology" (W. A. D. Anderson, ed.), Vol. 1, p. 35. C. V. Mosby Co., St. Louis (1971).

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