# The inflammatory response to carrageenan

It has been suggested by several authors that the inflammatory response to carrageenan is composed of a number of phases (Van Arman, Begany & others, 1965; Vinegar, Schreiber & Hugo, 1969; Di Rosa, Giroud & Willoughby, 1971; Garcia Leme, Hamamura & others, 1973), and that these phases can be affected independently by different drugs (Bhalla & Tangri, 1970; Di Rosa & others, 1971; Garcia Leme & others, 1973; Nakamura & Shimizu, 1974). Since, however, the conclusions of these authors conflict in a number of respects it was decided to re-examine the time course of carrageenan paw oedema in the rat with respect to paw swelling and protein extravasation.

Male Wistar rats (150–200 g) were maintained on standard laboratory diet with tap water freely available. Carrageenan paw oedema was induced by injection of 0·1 ml of 1·0% (w/v) carrageenan (Viscarin Marine Colloids) in 0·9% (w/v) sodium chloride in the plantar region of the right hind foot. Paw volumes were measured immediately after injection and at intervals thereafter using a mercury plethysmograph as described by Van Arman & others (1965). Results were expressed as the increase in paw volum in ml above the initial volume.

For studies on plasma protein extravasation, 1·0 $\mu$Ci of $^{125}$I labelled human serum albumin (Radiochemical Centre, Amersham) was injected intravenously into the tail vein of each rat 15 min before the injection of carrageenan. The radioactivities of the paws were determined at intervals by holding each rat, inserting the injected paw into the sample well of a gamma counter (Echo Electronics Ltd.) and determining the time required for 10 000 counts to be recorded on the scale. Lead screening was arranged so that radiation from the rest of the animal was minimal. Results were converted to counts $min^{-1}$ and expressed as the % increase above the initial reading. The radioactivity of the uninjected paw did not change during the experiments.

Carrageenan pleurisy was induced as described by Vinegar, Truax & Selph (1973). This involved injection of 0·25 ml of 0·2% (w/v) carrageenan in 0·9% (w/v) sodium chloride into the pleural cavity whilst the rats were lightly anaesthetized with ether. The animals were killed at intervals with ether, 1·0 ml of 0·9% (w/v) sodium chloride was injected intrapleurally, and the thorax gently massaged for 30 s. The chest wall was carefully cut open and the fluid recovered with a Pasteur pipette. Results are expressed as the weight of fluid recovered minus the weight of fluid injected i.e. 1·25 g.

Over a number of years we have consistently found that the increase in foot volume following subplantar injection of carrageenan consists of an early, transient phase, and a delayed, prolonged phase (Fig. 1A). This biphasic response is best detected by measurements made 25, 45, 85, 165 and 300 min after injection.

To determine to what extent this response was dependent on the trauma of injection as opposed to a specific response to carrageenan, the following experiments were performed.

(i) The response to carrageenan and the response to injection of the same volume of saline were compared (Fig. 1A). When the early transient response to saline is subtracted from the bi-phasic response to carrageenan, the early phase of carrageenan oedema is greatly reduced.

(ii) Groups of six rats were injected with varying amounts of carrageenan i.e. 0·5 1·0 or 2·0 mg in 0·1 ml of 0·9% (w/v) sodium chloride. Fig. 1B shows that although the delayed phase is increased by increasing the amount of carrageenan injected, the early phase is not.

(iii) The time course of carrageenan pleurisy was compared with the time course of carrageenan paw oedema. Injection of fluid into the pleural cavity causes less damage than injection into solid tissues like the paw, and despite the larger volume

injected (0·25 ml) there was no early phase in carrageenan pleurisy. When the subplantar response and the intrapleural response are plotted on the same axes (Fig. 1C), it can be seen that the single phase of the intrapleural reaction coincides with the delayed phase of the subplantar reaction.

The increase in paw volume and accumulation of radioactively labelled protein in the paws were measured following subplantar injection of carrageenan. As shown in Fig. 1D both parameters showed a bi-phasic response with identical time courses, indicating that plasma protein extravasation takes place throughout the reaction. A similar biphasic response was obtained when the animals were anaesthetized with

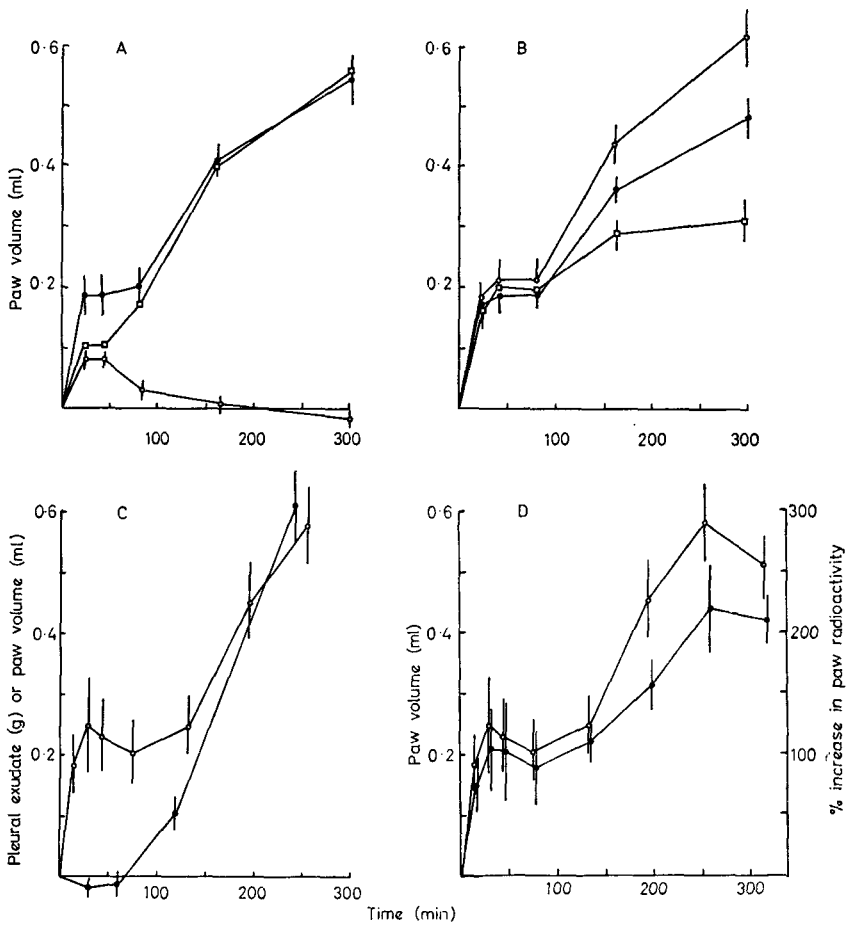


FIG. 1A. The increase in paw volume (ml) with time (min) following subplantar injection of: ● 1 mg of carrageenan in 0·1 ml of saline, and ○ 0·1 ml of saline. Each point is the mean of 6 rats ± s.e.m. □ represents the response to carrageenan after subtraction of the response to saline.

B. The increase in paw volume (ml) with time (min) following subplantar injection of: □ 0·5, ● 1·0 and ○ 2·0 mg of carrageenan in 0·1 ml of saline. Each point is the mean of 6 rats ± s.e.m.

C. Comparison of the quantities of exudate obtained on injection of carrageenan into the rat paw, with injection into the pleural cavity. ○—○ the response in ml to subplantar injection of 1 mg carrageenan in 0·1 ml of saline. ●—● the response in g to intrapleural injection of 0·5 mg carrageenan in 0·25 ml of saline. Each point is the mean of 6 rats ± s.e.m.

D. Comparison of the increase in paw volume (oedema) with the percentage increase in paw radioactivity ($^{125}$I labelled human serum albumin-protein accumulation) following subplantar injection of 0·1 mg of carrageenan in 0·1 ml saline. ○—Increase in paw volume (ml). ●—% increase in radioactivity. Each point is the mean of 5 rats ± s.e.m. A second independent experiment gave similar results.

pentobarbitone (i.p.) and the radioactivity of the paws was recorded continuously (not shown).

These results indicate that the early phase of the reaction to subplantar injection of carrageenan is due to the trauma of injection, since it is seen after injection of saline, it is independent of the quantity of carrageenan injected, and it is not seen following intrapleural injection. The same conclusion was reached by Van Arman & others (1965), on the basis of experiments similar to that shown in Fig. 1A.

Although a number of reports describe a biphasic change in paw volume (Van Arman & others, 1965; Vinegar & others, 1969; Nakamura & Shimizu, 1974) other reports show a single phase (Di Rosa & others, 1971; Winter, Risley & Nuss, 1962; Bolam, Elliott & others, 1974). The principal reason for this would seem to be that the early phase is transient and can only be detected by measurements made within one hour of the injection of carrageenan. In addition, Moore & Trottier (1974), in a comparison of the irritancy of different samples of carrageenan, only detected a biphasic response with the less irritant samples.

The results further indicate that protein extravasation occurs throughout the whole response to carrageenan (Fig. 1D). Garcia Leme & others (1973), on the basis of measurements of the rate of leakage of protein bound dye into the perfused subcutaneous space of carrageenan injected paws, claimed that the response to carrageenan was composed of an initial phase of increased permeability to plasma proteins followed by a phase of increased permeability to water alone. This conclusion was contested by Hurley & Willoughby (1973) who claimed that the order of these phases was reversed, and by Bolam & others (1974) who like us, claimed that protein extravasation took place throughout the reaction.

Closer scrutiny of the work of Garcia Leme & others (1973), however, reveals that they have compared the protein extravasation during discrete 40 min periods with the cumulative increase in foot volume, thus giving the incorrect impression that protein accumulation takes place only during the early phase of the reaction. If on the other hand cumulative protein extravasation is compared with increase in foot volume it can be seen that protein extravasation occurs throughout the reaction.

This work was supported by a grant from the Arthritis and Rheumatism Council.

*Department of Pharmacology,*
*Guy's Hospital Medical School,*
*London SE1 9RT, U.K.*

N. S. DOHERTY*
B. V. ROBINSON

May 12, 1975

* Department of Medical Chemistry, University of Turku, Kiinamyllynkatu 10, 20520, Turku 52, Finland.

## REFERENCES

BHALLA, T. N. & TANGRI, K. K. (1970). *J. Pharm. Pharmac.*, **22**, 721.

BOLAM, J. P., ELLIOTT, P. C. N., FORD-HUTCHINSON, A. W. & SMITH, M. J. H. (1974). *Ibid.*, **26**, 434–440.

DI ROSA, M., GIROUD, J. P. & WILLOUGHBY, D. A. (1971). *J. Path.*, **104**, 15–29.

GARCIA LEME, J., HAMAMURA, L., LEITE, M. P. & ROCHA E SILVA, M. (1973). *Br. J. Pharmac.*, **48**, 88–96.

HURLEY, J. V. & WILLOUGHBY, D. A. (1973). *Pathology*, **5**, 9–21.

MOORE, E. & TROTTIER, R. W. (1974). *Res. Comm. Chem. Path. Pharmac.*, **7**, 625–628.

NAKAMURA, H. & SHIMIZU, M. (1974). *Jap. J. Pharmac.*, **24**, 393–405.

VAN ARMAN, C. G., BEGANY, A. J., MILLER, L. M. & PLESS, H. H. (1965). *J. Pharmac. exp. Ther.*, **150**, 328–334.

VINEGAR, R., SCHREIBER, W. & HUGO, R. (1969). *Ibid.*, **166**, 96–103.

VINEGAR, R., TRUAX, J. F. & SELPH, J. L. (1973). *Proc. Soc. exp. biol. Med.*, **143**, 711–714.

WINTER, C. A., RISLEY, E. A. & NUSS, G. W. (1962). *Ibid.*, **111**, 544–547.