Effects of a human plasma fraction on skin reactions in the rat and rabbit

The human plasma fraction (1ml of the combined fractions II and IV, Ford-Hutchinson, Insley & others, 1973) that showed anti-inflammatory activity in the rat carrageenan-induced paw oedema test, when injected intravenously in female Wistar rats after the intradermal injection of 0·05 ml of 1·0 g per 100 ml (w/v) carrageenan (Viscarin Marine Colloids) in 0·9 g per 100 ml (w/v) NaCl, also inhibited the extravasation of circulating azovan (Evans) blue dye.

All intradermal injections were made on the backs of the animals, which were shaved 24 h before. The diameters of the lesions were measured on the under surface of the skin (Carr & Wilhelm, 1965), 3 h after injection of the dye, with a Bencard Skin Testing Reaction Gauge and the intensity of the extravasated dye was assessed with a Lovibond Flexible Optic Tintometer according to Chamberlin & Jolles (1972). The plasma fraction significantly reduced both the diameter and the intensity of the lesions (control: diam. 8·5 $\pm$ 1·33 mm, intensity, 76 $\pm$ 7%; plasma fraction: diam. 4·9 $\pm$ 2·1 mm, intensity 49 $\pm$ 18%. $P<0{\cdot}001$, n = 9). However, the plasma fraction did not affect the blue dye permeability produced by the intradermal injection of histamine (5 μg), 5-hydroxytryptamine (5 μg), bradykinin (0·5 μg) or prostaglandins E_1 (0·5 μg), E_2 (0·5 μg) or $F_{2\alpha}$ (0·5 μg). Thus the anti-inflammatory effect of the plasma fraction in the cutaneous reaction to carrageenan in the rat is not due to an interference with the actions of the above mediators. This finding confirms the results of other work with carrageenan-induced rat paw oedema (Bolam, Elliott & others, 1974; Smith, Ford-Hutchinson & others, 1974).

The effects of intravenous injections of the plasma fraction were also tested against the passive cutaneous anaphylaxis (PCA) and reversed passive Arthus reactions in the rat by the method of Levy (1964). The antigen in both reactions was bovine serum albumin (Sigma Chemical Co.), 8 mg per animal, and the antibody was rabbit anti-bovine serum albumin (Behringwerka A.G., Marburg), 0·05 ml (0·35 mg protein) per animal. The results show that while there was no effect on the PCA reaction (control: diam. 10·1 $\pm$ 1·7 mm, intensity 76 $\pm$ 7%; plasma fraction: 9·8 $\pm$ 1·7 mm, 77 $\pm$ 7% n = 10) the plasma fraction significantly reduced both the diameter of the skin lesion and the intensity of the dye infiltration in the Arthus reaction (control: diam. 11·2 $\pm$ 1·1 mm, intensity 84 $\pm$ 6%; plasma fraction: 7·9 $\pm$ 1·4 mm, 62 $\pm$ 8% respectively $P<0{\cdot}001$, n = 10). Further experiments were performed with the reversed passive Arthus reaction in the rabbit (Humphrey, 1955) using 20 mg of bovine serum albumin and 0·1 ml (0·7 mg protein) of antibody per animal. It was found that intravenous administration of the plasma fraction produced a small but definite reduction in the degree of haemorrhage at the site of intradermal challenge.

One of the main differences between cutaneous anaphylactic reactions, such as PCA, and the Arthus phenomenon is the role of leucocytes (Cochrane, 1965). The Arthus reaction is eliminated in the rat (Cochrane, 1965) by depletion of polymorphonuclear leucocytes whereas cutaneous anaphylaxis is not affected. The results of the present work therefore suggest that the plasma fraction may exert its anti-inflammatory action in cutaneous reactions, such as the Arthus reaction and carrageenan-induced lesion, by interfering with leucocyte emigration. This mechanism may also apply, at least in part, to other inflammatory responses, e.g. carrageenan-induced paw oedema reaction. However, one of the major effects of the plasma fraction in this latter reaction is to reduce the increased permeability to circulating proteins (Bolam & others, 1974) and there is evidence (Hurley, 1972) that leucocyte emigration

and increased vascular permeability are completely separable phenomena. If one of the sites of the anti-inflammatory effects of the plasma fraction involves leucocyte migration, then its effects resemble those of many conventional antirheumatic drugs (see Di Rosa, Papadimitriou & Willoughby, 1971). It has also been suggested that complement may be concerned in causing the accumulation of polymorphs in the Arthus reaction (Ward, 1968) as well as being an important mediator of non-immunological events leading to increased vascular permeability (Willoughby, Coote & Turk, 1969; Di Rosa, Giroud & Willoughby, 1971). The plasma fraction had no effect on the haemolytic complement system *in vitro* or on the circulating titre of total haemolytic complement (Rosenburg & Tachinana, 1962) of groups of rats killed either 30 min or 2 h after the intravenous injection of the fraction.

We wish to thank Abbott Laboratories Ltd., Beecham Research Laboratories Ltd., King's College Hospital and Medical School Research Committee, the National Research Development Corporation, the Nuffield Foundation and the Wates Foundation for financial support, and the Blood Transfusion Centre, Tooting, London, S.W.17 for the human plasma.

Department of Biochemical Pharmacology,
King's College Hospital Medical School,
Denmark Hill,
London SE5 8RX, U.K.

J. P. BOLAM
A. W. FORD-HUTCHINSON
P. N. C. ELLIOTT
M. J. H. SMITH

March 22, 1974

REFERENCES

BOLAM, J. P., ELLIOTT, P. N. C., FORD-HUTCHINSON, A. W. & SMITH, M. J. H. (1974). *J. Pharm. Pharmac.*, **26**, In the Press.

CARR, J. & WILHELM, D. L. (1965). *Nature, Lond.*, **208**, 653–655.

CHAMBERLIN, G. J. & JOLLES, B. (1972). *Methods in Microcirculation Studies*, pp. 18–27. London: H. K. Lewis.

COCHRANE, C. G. (1965). *The Inflammatory Process*, pp. 613–648. London: Academic Press.

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

DI ROSA, M., PAPADIMITRIOU, J. M. & WILLOUGHBY, D. A. (1971). *Ibid.*, **105**, 239–256.

FORD-HUTCHINSON, A. W., INSLEY, M. Y., ELLIOTT, P. N. C., STURGESS, E. A. & SMITH, M. J. H. (1973). *J. Pharm. Pharmac.*, **25**, 881–886.

HUMPHREY, J. H. (1955). *Br. J. exp. Path.*, **36**, 268–289.

HURLEY, J. V. (1972). *Acute Inflammation.* London: Churchill Livingstone.

LEVY, L. (1964). *Immunology*, **7**, 91–96.

ROSENBERG, L. T. & TACHINANA, D. K. (1962). *J. Immunol.*, **89**, 861–867.

SMITH, M. J. H., FORD-HUTCHINSON, A. W., ELLIOTT, P. N. C. & BOLAM, J. P. (1974). *J. Pharm. Pharmac.*, **26**, 690–697.

WARD, P. A. (1968). *Biochem. Pharmac. Supplement*, 99–105.

WILLOUGHBY, D. A., COOTE, E. & TURK, J. L. (1969). *J. Path.*, **97**, 295–305.