Short communications

Separation of irritancy from the anti-inflammatory component of inflammation exudate

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The anti-inflammatory component found in inflammation exudates has been purified by Sephadex gel filtration and preparative polyacrylamide electrophoresis. Highly purified material produces no greater irritancy upon injection than 0·9% sodium chloride.

Over the past few years inflammation exudates have been shown to inhibit a variety of inflammatory reactions, that is granulation tissue deposition (Di Pasquale, Girerd, Beach & Steinetz, 1963; Robinson & Robson, 1966) carrageenin oedema (Billingham, Robinson & Robson, 1969a, 1969b; Bonta, Bhargava & De Vos, 1970) and delayed hypersensitivity (Gaugus, Billingham & Rees, 1970).

Recent experiments of Atkinson, Boura & Hicks (1969) and Atkinson & Hicks (1971) have also shown that inflammation exudates are irritant when injected subcutaneously or into the foot of a rat. They further correlated this irritant activity with the degree of anti-inflammatory activity produced, and questioned the presence of a specific anti-inflammatory factor, explaining the action of exudate in terms of a non-specific counter-irritation of unknown mechanism.

Results presented in this paper show that by protein fractionation techniques it has been possible to obtain an anti-inflammatory material from exudate which is almost devoid of irritant properties.

Methods.—Inflammation exudates were obtained from the rat (male Sprague Dawley 200–300 g) by sponge implantation as described previously (Billingham *et al.*, 1969a). The anti-inflammatory properties of such exudates or of protein fractions derived chromatographically from them were assessed by the carrageenin paw oedema test of Winter, Risley & Nuss (1962) as described before (Billingham *et al.*, 1969a). The irritancy of exudates and of separated fractions were determined by the method used by Atkinson & Hicks (1971). Sephadex G.150 gel filtration was carried out as described previously by Billingham, Gordon & Robinson (1971), and the preparative polyacrylamide electrophoresis was performed by the method of Brownstone (1969). Essentially this consisted of preparing a 7·5% polyacryamide gel in Tris/borate buffer at pH 8·7 and applying to this 800 mg of the freeze-dried anti-inflammatory material from a Sephadex G.150 separation (dissolved in 40 ml of buffer). The buffer for both the gel and electrode compartment consisted of 0·0125 M Tris and 0·00625 M borate (pH 8·7). The dimensions of the gel column were diameter 14·3 cm and depth 5 cm, and electrophoresis was performed until completion (approx. 24 h) using 200 V and 400 mA. As the protein solution was eluted from the column its optical density was measured at 253 nm using a flow-through cell, and 10 ml samples were collected. The samples were then pooled according to the overall protein peaks indicated by the spectrophotometer. The four fractions thus obtained (see later) were forced dialysed, freeze dried and tested for both anti-inflammatory and irritant activity.

Results.—For the assay of anti-inflammatory activity exudates or protein fractions derived from them were injected subcutaneously into each of five rats (male Sprague Dawley, 140–180 g) which were simultaneously injected into the right hind paw with 0·1 ml of a 1% solution of carrageenin (Marine Colloids Inc.) in 0·9% sodium chloride. Anti-inflammatory activity was expressed as the percentage inhibition of paw swelling in the treated rats compared with a saline treated control group four hours after the injections (Table 1).

The irritancy of the exudate and protein fractions was determined after injection of 0·1 ml of solutions of such material (at the same concentration as used in the

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TABLE 1. *Anti-inflammatory and irritant activity of inflammation exudate and its derivative protein fractions*

Treatment	Anti-inflammatory activity				Irritant activity		
	Concentration of protein solutions used for injection (mg/ml)	Dose in carrageenin oedema assay (mg/kg)	% inhibition of foot oedema compared with saline treated controls	*P* value	Increase in foot volume (ml) produced on injection of 0·1 ml ± S.D.	% Reduction of irritation with increasing purification compared with exudate	*P* value
Unfractionated inflammation exudate	100	600	56	<0.001	0·44 ± 0·05		
Anti-inflammatory fraction following G.150 separation of inflammation exudate	17	100	52	<0.001	0·32 ± 0·06	27	<0.001
Anti-inflammatory fraction (fraction 3) following separation of G.150 material on polyacrylamide electrophoresis	1·4	8	55	$<<0.001$	0·07 ± 0·003*	98*	<0.001

* The increase in foot volume produced by injection of 0·9% saline in the same experiment was 0·05 ± 0·04 ml. This was not significantly different from the effect of polyacrylamide fraction.

anti-inflammatory assay) into one hind paw of similar groups of five rats, and was measured as the increase in paw volume (in ml) produced at 3 hours.

Table 1 shows that the unfractionated exudate was both anti-inflammatory and irritant, that is, it reduced carrageenin oedema, and in its own right induced paw swelling. This is in agreement with the findings of Atkinson & Hicks (1971). However, even with the low degree of purification achieved by G.150 gel filtration (6 times on a weight basis: 600 mg/kg–100 mg/kg) the irritancy of the exudate material was lessened whilst anti-inflammatory activity was retained in full.

Such material, when applied to polyacrylamide electrophoresis, separated into three main protein peaks. The first peak consisted mainly of albumin (fraction 1), the second of α_1 and α_2 glycoproteins (fraction 2) and the last of transferrin (fraction 4). Preceding the transferrin peak was a small additional peak (fraction 3), and it is this fraction which contained most of the anti-inflammatory activity.

This material produced a 55% inhibition of carrageenin oedema at a dose of only 8 mg/kg (representing a 75-fold purification on a weight basis when compared with crude exudate).

These results represent a single separation using polyacrylamide electrophoresis. However, varying the starting material, that is the particular fraction taken from the G.150 separation, altered slightly the protein distribution following polyacrylamide electrophoresis and hence the position of maximum anti-inflammatory activity. Only in the experiment described was irritancy of the active material measured. The degree of paw swelling induced upon injection of such material was no greater than that produced by 0·9% sodium chloride (see Table 1). In other words the irritancy had been lost whilst the anti-inflammatory activity was retained.

Discussion.—It is reasonable to conclude from the above results that as the anti-inflammatory activity is increasingly purified, irritant contaminants are progressively removed. Alternatively, following high purification the considerable reduction of protein material injected during an anti-inflammatory assay, that is only 8 mg/kg compared with 600 mg/kg of rude exudate, may simply account for the reduction of irritancy. Of course a combination of these factors may be involved.

Recently, it has been shown that the anti-inflammatory activity is the property of a protein which is synthesized by the liver of injured animals (Billingham *et al.*, 1971). From this site it is presumably carried in the blood (Billingham *et al.*, 1969a; Billingham, Gaugas & Robinson, 1970) to the site of inflammation where it is found in the inflammation exudate.

It seems unlikely therefore that the irritant property of crude exudates is connected with this anti-inflammatory protein. What is more probable is that the irritancy is due to other substances in the exudate, either enzymes or their breakdown products or the products of cell death all of which are well known to be present. On this basis it would seem worthwhile to use as highly purified material as possible in any attempts to elucidate mechanisms of action.

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