The presence of a prostaglandin-like substance in the skin of the plaice, *Pleuronectes platessa* L.

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Fletcher & Baldo (1974) observed that the intradermal injection of fungal extracts which precipitate with human C-reactive protein and normal plaice serum caused an immediate (Type 1) hypersensitivity reaction in the skin of plaice, *Pleuronectes platessa* L. This communication reports the results of experiments designed to identify the pharmacological mediators responsible for this cutaneous anaphylaxis.

Skin from freshly killed plaice was chopped, washed and incubated with a specific plaice ringer (Cobb, Fox & Santer, 1973) in the presence of an extract of the dermatophyte fungus, *Epidermophyton floccosum*. Samples were withdrawn after incubation at room temperature and tested for smooth muscle activity on the following isolated preparations: gerbil and rat colon, rat duodenum and stomach strip and guinea-pig trachea and ileum. The extract contracted all tissues but the rat stomach strip preparation gave the most reproducible and dose-dependent responses and so was used to assay the active principle from the skin in subsequent experiments.

The fungus alone had no smooth muscle stimulating activity but extracts of skin which had not been challenged with the fungus exhibited a low level of activity.

The contractile response of the rat stomach strip to skin extract persisted even in the presence of antagonists of acetylcholine, histamine and 5-hydroxytryptamine and also following incubation with chymotrypsin, which destroys activity due to bradykinin.

Indomethacin (10–1000 μg/kg i.p.) injected into plaice 2 h before killing, produced a dose-dependent inhibition of the release of active material from both challenged and non-challenged skin. The activity could be partitioned from an acid aqueous phase into chloroform, from there into Krebs solution (pH 7) and finally back into chloroform at pH 3. This behaviour is consistent with the properties of a prostaglandin.

Following extraction, characterization of the prostaglandin-like activity was attempted using preparative thin-layer chromatography. In six different solvent systems, the pharmacological activity co-chromatographed almost exclusively with authentic E_2, the remainder (<5%) with prostaglandin F. Release of active material from challenged and non-challenged skin was then assayed by the bracketing technique on the rat stomach strip in equivalents of PGE_2. The extract from challenged skin produced a significantly ($P < 0.05$) greater response with incubation times between 20–80 minutes. There was no significant difference in the responses after 120 min incubation.

An immediate hypersensitivity reaction resembling that induced by *Epidermophyton floccosum* resulted from the intradermal injection of PGE_2 (100 ng/ml). Indomethacin did not however completely inhibit the fungal skin reaction *in vivo*, suggesting that other mediators may also be involved.

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Stimulation of platelets and macrophages by carrageenin

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Carrageenin, a sulphated polysaccharide from green algae, induces hypotension when injected intravenously (Di Rosa & Sorrentino, 1970) and subplantar administration evokes a marked inflammatory response (Winter, Risley & Nuss, 1962). Different carrageenins vary in their inflammatory and hypotensive potency (Di Rosa, 1972) but the reasons for this are not clear. Since secretion by macrophages and platelets has been implicated in inflammatory responses, we have compared the activity of 4 carrageenin preparations as stimulants of blood platelets and macrophages *in vitro*.

Macrophage culture medium (CM) consisted of 'Autopow' minimum essential medium (Flow

Laboratories), supplemented with 2 mM glutamine, 20 mM HEPES buffer, 5 mM sodium bicarbonate, 50 μg/ml gentamycin and 20% (v:v) heat inactivated foetal bovine serum. Macrophages were obtained by peritoneal lavage of mice 3–4 days after injection of 1 ml thioglycollate medium. Cells were washed twice with phosphate buffered saline (PBS), resuspended in CM and incubated in multiwell plates at 37°C for 3–4 hours. Dead and non-adherent cells were removed by rinsing twice with PBS and macrophages were maintained in CM. Platelet aggregation and release of 5-hydroxytryptamine (5-HT), lysosomal enzymes (B-N-acetylglucosaminidase) and adenine were measured in 0.1 ml volumes of human platelet-rich plasma (PRP) and plasma-free platelet suspensions as previously described (Corey, Gordon, MacIntyre & Salzman, 1977). Plasma-free platelet suspensions were prepared by gel filtration (Salzman, Lindon & Rodvien, 1976). For estimation of macrophage secretion B-N-acetyl-glucosaminidase activity was measured fluorimetrically as for platelets and lactate dehydrogenase (LDH) activity was measured photometrically (Wroblewski & La Due, 1955).

Gelcarin, Iota, Kappa and Lambda carrageenins (100 μg/ml) stimulated macrophage lysosomal enzyme secretion after 36 h incubation; little release was observed after 14 hours. There was no significant leakage of the cytoplasmic enzyme LDH after 36 hours. The rank order of potency of the carrageenins was Iota > Lambda > Gelcarin > Kappa. All the carrageenins induced platelet aggregation showing the same order of potency as for macrophage secretion. Aggregation in PRP, but not in gel-filtered platelets, was preceded by a large increase in optical density, but since this was also observed in platelet poor plasma it presumably represents an alteration in plasma proteins. The aggregation response in citrated PRP consisted of two phases, the second of which was associated with plasma proteins. The aggregation response in citrated PRP consisted of two phases, the second of which was release of platelet granule constituents. This was a selective process—less than 10% release of the cytoplasmic marker, adenine, accompanied release of 70% 5-HT (dense granule constituent) and 35% B-N-acetyl-glucosaminidase (α granule constituent).

Aspirin (1 mM) inhibited only the second phase of aggregation and the associated release of 5-HT and B-N-acetyl-glucosaminidase. Both phases of aggregation were inhibited by prostaglandin E_1 (1 μM) and aminophylline (5 mM), which elevate cellular cyclic AMP concentrations; by EDTA (1 mM) and EGTA (1 mM), which chelate divalent cations; and by quinacrine (10–50 μM), which inhibits phospholipase A_2 (Flower & Blackwell, 1976).

Carrageenin—induced platelet aggregation in heparinized PRP and gel-filtered platelets consisted of only one phase which was not associated with secretion of granule constituents.

In summary, the rank order of potency of the 4 carrageenins at stimulating secretion from macrophages and blood platelets *in vitro* was the same, and this is in accord with their relative potency *in vivo* (McCandless, 1965). There appears to be a common structural requirement for carrageenin activity as a secretory stimulant *in vitro* and as an inflammatory agent *in vivo*. Further studies *in vitro* should help to elucidate how inflammatory lesions are induced by carrageenin *in vivo*.

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