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 Samples were withdrawn after incubation at room temperature and tested for smooth muscle activity on the following isolated preparations: gerbil and rat colon, rat duodenum and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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References

COBB, J.L.S., FOX, N.C. & SANTER, R.M. (1973). A specific ringer solution for the plaice (*Pleuronectes platessa* L.). *J. Fish Biol.*, **5**, 587–591.

FLETCHER, T.C. & BALDO, B.A. (1974). Immediate hypersensitivity responses in flatfish. *Science*, **185**, 360–361.

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