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16. The microtubules which were observed to originate from the midlateral aspect of the isolated basal bodies which had been incubated with brain tubulin in vitro may be assembling onto the distal connecting fiber. This fiber between the two basal bodies has been described as the in situ site of attachment for the submembrane microtubules. However, in order to determine if this fiber is serving as an in vitro assembly site for microtubules, thin sectioning and electron microscopy will be required. Some basal bodies were observed that supported tubulin assembly from both distal and proximal ends, but not from their midlateral aspects. In these cases the presumptive microtubule assembly sites associated with the distal connecting fiber may be missing.
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Immediate Hypersensitivity Responses in Flatfish

Abstract. *Fungal extracts that precipitate with human C-reactive protein caused immediate erythema on subdermal injection into marine flatfish. Only species with calcium-dependent serum precipitins to these fungi showed skin reactions. Immediate hypersensitivity in a nonreactive species could be induced after injection with serum from reactive species. The transferable serum factor (or factors) was heat sensitive.*

In recent years phylogenetic aspects of antibody structure and function in poikilotherms have received a great deal of attention (*1*, *2*), but little attempt has been made to study the phylogeny of immediate and delayed hypersensitivity reactions. There have been a few attempts to demonstrate immediate hypersensitivity reactions in fish. These studies were based on methods that were designed to demonstrate systemic or passive cutaneous anaphylaxis, but results have been inconclusive (*1*, *3*).

Using some marine teleost species belonging to the order Heterosomata (flatfish), we have found that intradermal injections of some fungal extracts produce immediate (type 1) skin reactions (*4*) in the injected fish. Flatfish were selected for examination since, in many respects, they are ideal experimental animals for a study of immediate hypersensitivity in poikilotherms. Plaice, *Pleuronectes platessa* L., and the closely related flounder, *Platichthys flesus* (L.), are readily available throughout the year and are easily maintained in the aquarium. Both species are easy to handle, the accessible caudal vein is suitable for intravenous injection and blood collection, and, most important, skin reactions can be clearly observed on their nonpigmented under surfaces.

Fish used in our study were seinenetted in shallow water off the Aberdeenshire coast and transferred to aerated seawater tanks where they were maintained at 11° to 14.5°C. Experiments were generally carried out on fish that had been in the aquarium for periods ranging from 24 hours to 16 months, but a few fish were examined within 1 hour of capture. Both male and female fish, varying in age from 1 to 10 years, were used. Extracts of the fungi *Aspergillus fumigatus, Candida albicans, Epidermophyton floccosum, Micropolyspora faeni, Trichophyton mentagrophytes,* and *Trichophyton rubrum* and an extract from the house dust mite *Dermatophagoides farinae* were prepared from culture filtrates and whole mites by methods already described (*5*). These preparations are widely used as allergens in human skin tests.

The extracts and a peptido-polysaccharide isolated from *E. floccosum* (*6*) were injected (0.2 ml) into the skin of plaice at concentrations of 10 mg/ml in 0.19*M* NaCl. Extracts of *A. fumigatus, E. floccosum, T. mentagrophytes,* and *T. rubrum* and *E. floccosum* peptido-polysaccharide produced immediate erythema reactions in the skins of 69 of 70 plaice tested (Fig. 1). The one plaice that failed to react did show a slight response to *T. mentagrophytes* extract. In general, skin reactions were most pronounced when *E. floccosum* whole extract and *E. floccosum* peptido-polysaccharide were injected, but no skin reactions appeared after the injection of *E. floccosum* extract from which the peptido-polysaccharide had been removed with concanavalin A (*6*). No skin reactions were observed when plaice were injected with saline or with extracts from

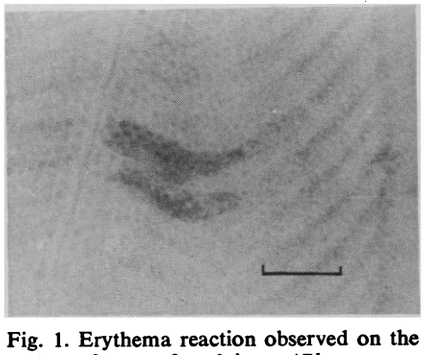

Fig. 1. Erythema reaction observed on the undersurface of plaice (*Pleuronectes platessa*) 5 minutes after subdermal injection of 0.2 ml of *Epidermophyton floccosum* extract (10 mg/ml in saline). The bar is equivalent to 0.75 cm.

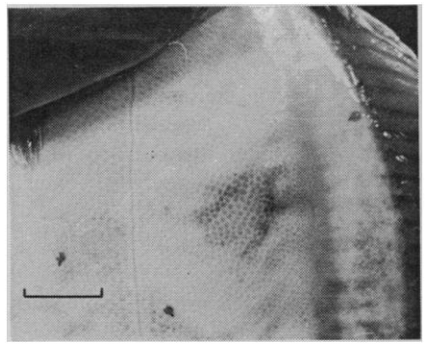

Fig. 2. Erythema reaction observed on the undersurface of flounder (*Platichthys flesus*) 1 hour after subdermal injection of 0.2 ml of *Epidermophyton floccosum* extract (10 mg/ml in saline). Flounder injected intravenously 24 hours earlier with plaice serum (1.5 ml serum per 100 g of the body weight of the flounder). The bar is equivalent to 2 cm.

C. albicans, M. faeni, or *D. farinae.* Skin reactions to *E. floccosum* extract were also observed in other flatfish: turbot, *Scophthalmus maximus* (L.), brill, *Scophthalmus rhombus* (L.), and dab, *Limanda limanda* (L.). We did not observe erythema reactions to *E. floccosum* extract or to any of the other extracts in 30 separate *P. flesus* tested over a 9-month period.

This result did, however, provide us with a suitable system with which to study the nature of the immediate erythema response observed in plaice. Within 3 hours of collection, plaice serum containing approximately 2.9 percent protein (*7*) was injected intravenously into flounder at a dose of 1 to 1.5 ml per 100 g of body weight. Twenty-four hours later the fish were challenged intradermally with 0.2 ml of *E. floccosum* extract. Skin reactions showing erythema and accompanied by swelling developed over the first hour (Fig. 2). Heating the plaice serum for 30 minutes at 56°C prior to transfer did not inhibit the flounder skin reaction, but heating for 4 hours at 56°C appeared to destroy the transferable factor (or factors).

Transfer of the immediate skin reaction to flounder was also demonstrated using passive cutaneous anaphylaxis (PCA) and Prausnitz-Küstner (P-K) methods (*8*). Intradermal injections (0.2 ml) of unheated or heated (56°C for 4 hours) plaice serums and of saline were given to flounders; 3 hours later 1 percent Evan's blue (1 ml) was given intravenously and this dose was followed 0.5 hour later by injection of 10 mg of *E. floccosum* extract. Significant bluing was observed at the sites where unheated serum had been injected. Similar results were obtained when intravenous challenge was made 24 hours after the intradermal serum injections. No bluing was seen at the sites of the saline or heated serum injections or at the sites of serum injections after the intravenous injection of dye and *C. albicans* extract or dye and *M. faeni* extract, both at 10 mg/ml. Extract of *E. floccosum* (10 mg/ml in saline) was also injected intradermally into flounders 3 to 24 hours after the intradermal injection of plaice serum. An immediate erythema reaction was observed when the fungal extract was injected at the same site as the plaice serum but not when injected at sites not previously exposed to plaice serum. No erythema was observed when saline, *C. albicans, M. faeni,* or *D. farinae* extracts were injected at the serum sites.

All the above experiments confirmed that the immediate skin reaction observed in plaice after the injection of *E. floccosum* extract can be transferred to the flounder by a factor (or factors) in plaice serum. These results indicate that the immediate skin reaction seen in plaice is a true, immediate (type 1) hypersensitivity reaction.

In humans it is now apparent that the reaginic or tissue-fixing antibodies belong to a specialized immunoglobulin class designated IgE (*9*). Other species —including the monkey, rabbit, guinea pig, rat, mouse, and dog—have also been shown to produce antibodies capable of attaching to target cells of the same species or of different species (or both) and releasing pharmacologically active agents from the cells (*10*). Comparable antibodies have not so far been described in poikilotherms, but the report by Cohen *et al.* (*11*) of anaphylactic reactions in frogs after injection of *Salmonella typhosa* preparations suggests that such antibodies probably exist in at least some poikilothermic species. The skin reactions we observed in flatfish appear to be specific for extracts that contain components which precipitate with human C-reactive protein (CRP).

We have found that plaice serums also contain precipitins which react with the C-substance–like constituents in *A. fumigatus, E. floccosum, T. mentagrophytes,* and *T. rubrum* and that these CRP-like precipitins migrate in the α_2 region and not in the β region of induced antibodies (*6, 12*). The C-substance–like activity and the skin-sensitizing action of culture filtrate extract from *E. floccosum* are associated with the peptido-polysaccharide isolated from the whole extract by reaction with concanavalin A (*6*). Of the five species of flatfish we examined, only the flounder lacked serum precipitins to the C-substance–like components of the fungi and only the flounder failed to show skin reactions when these extracts were injected intradermally. The component or components of plaice serum responsible for the immediate skin reactions described above may be a tissue-fixing antibody not so far detected as a precipitin, but the possibility remains that the skin reactions are mediated via the CRP-like protein in plaice serum. To our knowledge, an association between immediate cutaneous reactions and the presence of CRP in serum has yet to be established.

Human CRP shows extreme specificity for phosphorylcholine (*13*), and the determinant on the *E. floccosum* peptido-polysaccharide responsible for the reaction with plaice serum also appears to be phosphorylcholine (*6*). It seems then that the immediate reactions observed in flatfish are due either to the presence of as yet undescribed tissue-fixing antibodies or to CRP-like proteins which are capable of binding to cell surfaces and which, in the presence of C-substance–like compounds, are capable of releasing pharmacologically active substances. The binding to tissues of proteins with specificity for phosphorylcholine seems possible since phosphorylcholine antigens probably occur in lecithins and sphingomyelins, both of which are found in the membranes of animal tissues (*14*).

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