Inhibition of Immediate Hypersensitivity Responses in Flatfish

We recently showed that the intradermal injection of fungal extracts which precipitate with human C-reactive protein (CRP) and 'normal' plaice serum[1] cause an immediate (type I) hypersensitivity reaction in the skin of plaice, *Pleuronectes platessa* L.[2]. Although cutaneous anaphylaxis was not observed when the fungal extracts were injected into the closely related flounder, *Platichthys flesus* (L.), an erythema reaction could be induced in the skin of this species following i.v. injection of plaice serum and subsequent intradermal challenge with the fungal preparations[2]. The nature of the skin-fixing plaice serum factor(s) and the pharmacological mediators responsible for the cutaneous anaphylaxis in plaice and flounder have still to be established.

In this communication we report results of experiments designed to provide preliminary information on the pharmacologically-active agents involved in the immediate hypersensitivity responses of flatfish. In particular, we set out to investigate whether the poikilotherm skin reactions could be produced by injection of vasoactive substances and a histamine liberator and whether the skin reactions could be inhibited by some of the compounds which are effective in preventing type I hypersensitivity reactions in higher vertebrates.

In both plaice and flounder, immediate erythema reactions were produced following intradermal injection of 0.2 ml of histamine (5 mg/ml), serotonin (5 mg/ml) and the histamine liberator, compound 48/80 (500 µg/ml). The skin reactions induced by each of these preparations appeared identical with the reactions produced following injection of the fungal extracts[2]. We saw no obvious signs of distress in plaice following the i.v. injection of histamine (7 mg/100 g weight of fish) or *Epidermophyton floccosum* extract (10 mg/100 g) although the plaice showed an active cutaneous response to the intradermal injection of these substances. Intravenous injection of 20 mg histamine/100 g, however, proved lethal to plaice.

Plaice were used in experiments with drugs known to affect the action of vasoactive substances. Intravenous injection of the serotonin antagonist methysergide hydrogen maleate (40 µg/100 g) did not inhibit the skin reaction to 0.2 ml of *E. floccosum* extract (10 mg/ml) or to serotonin, but the antagonist proved toxic to plaice which died within 2 h of administration of the drug. Plaice were also sensitive to antihistamines such as promethazine HCl. Intravenous injection of 3 mg/100 g body weight killed the fish in less than 1 h and did not inhibit the skin reactions to fungal extracts or to histamine. Similar results were obtained with mepyramine maleate. Intravenous injection of the general anti-allergic agent pheniramine *p*-aminosalicylate (4 mg/100 g) however, completely inhibited the skin reaction of plaice to *E. floccosum* extract and reduced the reaction to histamine in 70% of the plaice tested. The most clear cut results obtained with the inhibition of the immediate skin reactions in plaice to *E. floccosum* extract and compound 48/80 were observed after the i.v. injection of disodium cromoglycate (DSCG). This compound is known to inhibit mast cell degranulation in some species[3] and has recently been shown to inhibit cyclic AMP phosphodiesterases in vitro[4]. Various concentrations of DSCG were injected i.v. into plaice and a dose of 16 mg/100 g, when given 1 min before intradermal injection of *E. floccosum* extract or compound 48/80, resulted in the complete inhibition of the erythema reactions. Inhibitory effects to skin challenge persisted for up to 18 h. A dose of 8 mg DSCG/100 g did not prevent the skin reactions but reduced their intensities.

Since the results with antihistamines and the serotonin antagonist proved inconclusive, it seemed possible that vasoactive substances other than histamine and serotonin might be involved in the plaice skin reactions. In mammals, an important but little understood mediator of anaphylaxis is slow reacting substance of anaphylaxis (SRS-A)[5,6]. Reports that the antifilarial chemotherapeutic diethylcarbamazine citrate can suppress antigen-induced release of SRS-A in the rat and from monkey lung[7,8], prompted us to use this drug in in vivo experiments with plaice. Intravenous injection of 6 mg of diethylcarbamazine citrate/100 g, less than 5 min before challenge with *E. floccosum* extract, completely inhibited the immediate erythema reaction and substantially reduced the reaction to compound 48/80. When the drug was given 15 min before challenge the intensity of the erythema was reduced but not abolished. A short duration of action of diethylcarbamazine was also noticed by ORANGE et al.[7] in their studies with rats. The similarity between fish and mammals in the action of diethylcarbamazine was not reflected with DSCG. Our finding of the long term action of DSCG in plaice was at variance with mammalian results where the inhibitory action was of short duration and where DSCG and antigen had to be presented together for effective inhibition to occur[9].

In higher vertebrates it is now apparent that the observed symptoms of an immediate hypersensitivity reaction are caused by the release of mediators from target cells[10,11]. The mediators, which have a profound effect on smooth muscle and vascular permeability, are released from cells already presensitized by the presence of tissue-fixing or reaginic antibodies on their surfaces[12]. The target cells appear to be composed largely of mast cells and basophils but release of mediators can be induced in some mammalian species by allergen challenge of reagin-sensitized lung tissue, nasal polyps and peripheral blood leukocytes[13]. Mast cells have been described in teleosts[14] and demonstrated in plaice skin[15]. The eosinophilic granular cells described by ROBERTS et al.[15] in the plaice, might also be involved as target cells, since following intradermal injection of compound 48/80 they became active and were observed migrating from their usual basal position in the epidermis. Within 1 h of injection, the epidermis appeared depleted of these cells in areas of cutaneous anaphylaxis (C.K. MURRAY and T.C.F., in preparation). Histamine is known to occur in

[1] B. A. BALDO and T. C. FLETCHER, Nature, Lond. *246*, 145 (1973).
[2] T. C. FLETCHER and B. A. BALDO, Science *185*, 360 (1974).
[3] T. S. C. ORR, D. E. HALL, J. M. GWILLIAM and J. S. G. COX, Life Sci. *10*, 805 (1971).
[4] A. C. ROY and B. T. WARREN, Biochem. Pharmac. *23*, 917 (1974).
[5] R. P. ORANGE and K. F. AUSTEN, Adv. Immun. *10*, 105 (1969).
[6] W. E. BROCKLEHURST, Proc. R. Soc. Med. *66*, 1198 (1973).
[7] R. P. ORANGE, M. D. VALENTINE and K. F. AUSTEN, Proc. Soc. exp. Biol. Med. *127*, 127 (1968).
[8] T. ISHIZAKA, K. ISHIZAKA, R. P. ORANGE and K. F. AUSTEN, J. Immun. *106*, 1267 (1971).
[9] D. S. THOMSON and D. P. EVANS, Clin. exp. Immun. *13*, 537 (1973).
[10] K. AAS, *The Biochemical and Immunological Basis of Bronchial Asthma*, (C. C. THOMAS, Springfield, Ill, 1972).
[11] L. M. LICHTENSTEIN, *Clinical Immunobiology* (Eds. F. H. BACH and R. A. GOOD; Academic Press, New York 1972), vol. 1, p. 243.
[12] D. R. STANWORTH, *Immediate Hypersensitivity*, (North-Holland Publ. Co., Amsterdam 1973).
[13] J. A. GRANT and L. M. LICHTENSTEIN, J. Immun. *112*, 897 (1974).
[14] E. KAPA and G. CSABA, Acta biol. hung. *24*, 19 (1973).
[15] R. J. ROBERTS, H. YOUNG and J. A. MILNE, J. Fish Biol. *4*, 87 (1972).

fish[16–18] and the presence of this biologically active amine, but not 5-hydroxytryptamine, has been demonstrated in the dermis of plaice[15]. Although SRS-A has been demonstrated in primate skin[19], its cellular origin remains obscure and there appear to be no reports of finding SRS-A in any tissues from poikilotherms.

Although the mechanisms involved in immediate hypersensitivity reactions have been the subject of intensive study[20–22], much has still to be learned about the chemical mediators involved and this is especially true of mediators found in mammals other than primates. The mediators, target cells and sensitivities of different tissues vary with species[11,23] and with poikilotherms the available knowledge on all of these aspects is extremely limited.

In our experiments the administration of antihistamines alone did not inhibit the plaice erythema reactions. This may indicate that histamine is not involved in the plaice skin reactions or that mediators other than histamine are also involved. However, inhibition of the fungal extract – and compound 48/80 – induced plaice erythema reactions by DSCG and diethylcarbamazine, compounds known to inhibit some type I allergic responses, supports our earlier conclusion[2] that we are dealing with a true immediate hypersensitivity reaction in a poikilotherm. Further experiments are in progress in an attempt to identify the individual pharmacologically-active compounds and the target cells which are involved in the flatfish cutaneous reactions.

Résumé. Comme le cromoglycate disodique et la diéthylcarbamazine (qui ne sont pas des antistaminiques) inhibent la réaction d'hypersensibilité immédiate chez *Pleuronectes platessa* à la suite d'une injection intradermique d'extraits fungiques, il semble que les médiateurs autres que l'histamine peuvent être impliqués.

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[16] C. VEIL, Acta physiol. pharmac. néerl. *6*, 386 (1957).
[17] O. B. REITE, Physiol. Rev. *52*, 778 (1972).
[18] W. LORENZ, E. MATEJKA, A. SCHMAL, W. SEIDEL, H.-J. REIMANN, R. UHLIG and G. MANN, Comp. gen. Pharmac. *4*, 229 (1973).
[19] B. KURITZKY and L. GOODFRIEND, Int. Archs Allergy appl. Immun. *46*, 552 (1974).
[20] R. P. ORANGE, M. A. KALINER and K. F. AUSTEN, *Biochemistry of the Acute Allergic Reactions* (Eds. K. F. AUSTEN and E. L. BECKER; Blackwell Scientific Publ., Oxford 1971), p. 189.
[21] E. L. BECKER and P. M. HENSON, Adv. Immun. *17*, 93 (1973).
[22] H. R. BOURNE, L. M. LICHTENSTEIN, K. L. MELMON, C. S. HENNEY, Y. WEINSTEIN and G. M. SHEARER, Science *184*, 19 (1974).
[23] J. L. MONGAR and H. O. SCHILD, Physiol. Rev. *42*, 226 (1962).
[24] N.E.R.C. Institute of Marine Biochemistry, St. Fittick's Road, Aberdeen AB1 3RA (Scotland), to whom correspondence should be addressed.

Hexose-6-Phosphate Dehydrogenase from Human Tissues: an Electrophoretic Study in Health and Disease[1]

Hexose-6-phosphate dehydrogenase (H6PD; EC 1.1.1.47) is a microsomal enzyme which has been demonstrated in a number of tissues from different species[2–12]. It is identical with glucose dehydrogenase[5,13,14]. After biochemical and immunological experiments, and hybridization studies in trout, it was suggested that H6PD and glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) arose from a common ancestral type of G6PD, an interesting relationship of H6PD and G6PD on the evolutionary basis has been established[15–18]. In mammalian tissues considerable biochemical differences between H6PD and G6PD have been found[3–5,8,11,12]; a comparison of the two enzymes is given in the Table. Electrophoretic studies on human liver[3] and placenta[9] suggested that H6PD is a highly polymorphic enzyme. However, no distinct pattern of inheritance could be defined by the results reported. We have performed an electrophoretic study

Comparison of hexose-6-phosphate-dehydrogenase (H6PD) and of glucose-6-phosphate-dehydrogenase (G6PD)

Properties	H6PD	G6PD
Localization	Microsomal fraction	Soluble fraction
Genetic control	Autosomal	*X*-chromosomal
Substrates	G6P	G6P
	Gal-6P	(2-d-G6P)
	2-d-G6P	
	Glucose	
Coenzymes	NADP	NADP
	NAD	(NAD)
Molecular weight	223,000	102,000
Binding to CM-cellulose	+	+++
Electrophoretic mobility	slow	fast
Precipitation by anti-G6PD	—	+
Inactivation by anti-G6PD	—	+

[1] This paper contains a considerable part of the doctoral thesis of G. M. SCHMIDT.
[2] C. R. SHAW and E. BARTO, Science *148*, 1099 (1965).
[3] C. R. SHAW, Science *153*, 1013, (1966).
[4] S. OHNO, H. W. PAYNE, M. MORRISON and E. BEUTLER, Science *153*, 1015, (1966).
[5] E. BEUTLER and M. MORRISON, J. biol. Chem. *242*, 5289 (1967).
[6] C. R. SHAW and L. KOEN, Ann. N.Y. Acad. Sci. *151*, 149 (1968).
[7] S. K. SRIVASTAVA and E. BEUTLER, J. biol. Chem. *244*, 6377 (1969).
[8] B. MANDULA, S. K. SRIVASTAVA and E. BEUTLER, Arch. Biochem. Biophys. *141*, 155 (1970).
[9] J. F. HUDDLESTON, G. LEE and J. C. ROBINSON, Am. J. Obstet. Gynec. *109*, 1017 (1971).
[10] J. B. SHATTON, J. E. HALVER and S. WEINHOUSE, J. biol. Chem. *246*, 4878 (1971).
[11] H. KIMURA and M. YAMASHITA, J. Biochem. *71*, 1009 (1972).
[12] S. K. SRIVASTAVA, K. G. BLUME, E. BEUTLER and A. YOSHIDA, Nature New Biol. *238*, 240 (1972).
[13] R. P. METZGER, S. S. WILCOX and A. N. WICK, J. biol. Chem. *239*, 1769 (1964).
[14] R. P. METZGER, S. S. WILCOX and A. N. WICK, J. biol. Chem. *240*, 2767 (1965).
[15] J. J. STEGEMAN and E. GOLDBERG, Biochem. Genet. *5*, 579 (1971).
[16] J. J. STEGEMAN and E. GOLDBERG, Biochem. Genet. *7*, 279 (1972).
[17] J. J. STEGEMAN and E. GOLDBERG, Comp. Biochem. Physiol. *43*, 241 (1972).
[18] T. YAMAUCHI and E. GOLDBERG, Biochem. Genet. *10*, 121 (1973).