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Identification of glycoproteins in goblet cells of epidermis and gill of plaice (Pleuronectes platessa *L.*), *flounder* (Platichthys flesus (*L.*)) *and rainbow trout* (Salmo gairdneri *Richardson*)

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Synopsis. A quantitative analysis has been made of the glycoproteins present in the goblet cells of the epidermis, gill filaments and gill lamellae of three species of teleost fish. The glycoproteins have been identified by a combination of techniques, including the use of the enzyme sialidase followed by Alcian Blue staining, at pH 2.6 or 1.0, in combination with periodic acid–Schiff. The selected fish were representative of species living in marine, freshwater and estuarine environments.

The range of glycoproteins identified in these fish was similar to that found in mammalian tissue in that both neutral and acid glycoproteins were present, the latter included both sialomucins sensitive and resistant to sialidase, and sulphomucin. A single goblet cell contained either neutral or acid glycoproteins alone or in combination. Only the epidermis of the plaice and rainbow trout contained uniform cell populations producing acid glycoproteins, the former sulphomucin and the latter mainly sialomucin. At each site in the flounder and in the gill epithelia of the plaice and rainbow trout, the goblet cell population was mixed, with cells producing each type of glycoprotein. The number of goblet cells producing each type of glycoprotein varied at each tissue site.

Introduction

Mucous secretions of fish are usually assumed to be protective (Jakowska, 1963) but experimental evidence to support this is not always readily available. The physical measurements of Rosen & Cornford (1971), however, clearly indicate friction reducing properties of cutaneous mucus from certain fish, while the presence of antibodies (Fletcher & Grant, 1969) and the bacteriolytic enzyme lysozyme (Murray & Fletcher,

1976), in plaice mucus, indicate that it is the vehicle for biologically active molecules. Hughes & Wright (1970) described the mucous film covering the secondary gill lamellae in teleost and elasmobranch fishes and suggested that it might have an important function in relation to gas, ionic and water exchanges at the gill surface.

Histochemical studies have demonstrated that goblet cells from the epidermis and gills of many teleosts contain glycoproteins (Asakawa, 1970; Bremer, 1972; Zaccone, 1972, 1973; Harris *et al.*, 1973; Carmignani & Zaccone, 1974; Ojha & Munshi, 1974) but these studies have been confined to cells of either the skin or of the gill epithelium and usually no comparison has been made in the same fish between the glycoproteins from these two regions (Porcelli & Novelli, 1970).

Pickering (1974) has counted mucous cells in the skin of the brown trout and char and related the goblet cell numbers to the sialic acid content of the skin. However, no quantitative analyses of the glycoprotein types in fish epithelia have been made, and at present, histochemistry offers a more sensitive method for localizing the different types of glycoproteins, and their intracellular combinations, than the biochemical analysis of tissue or of removed mucus. The methods of Alcian Blue (AB) staining developed for mammalian epithelial acid glycoproteins have been used to analyse the different types of glycoproteins in human and pig bronchial submucosal gland (Jones & Reid, 1973*a*,*b*; Jones *et al.*, 1975) and have been employed in the present study to identify the types of epithelial glycoproteins occurring in fish skin and gill.

Three teleost fish were selected for study: the plaice, *Pleuronectes platessa* L., the flounder, *Platichthys flesus* (L.) and the rainbow trout, *Salmo gairdneri* Richardson. These fish were representative of a salt, estuarine and a freshwater environment respectively. This paper describes the epithelial glycoproteins in goblet cells of the epidermis, gill filament and secondary lamellae of normal (i.e. untreated) fish and is the basis for a comparative study (Jones, Fletcher & Reid, in preparation) of the glycoproteins of plaice and rainbow trout exposed to pollutants, heavy metals and detergents in particular, and of the effect of variation in salinity on the synthesis of glycoproteins in flounders adapted to fresh or saltwater environments.

Materials and methods

Plaice and flounder, the flatfish, were seine-netted in shallow water off the Aberdeen coast and transferred to aerated seawater tanks in an aquarium. Rainbow trout were obtained from Howietown and Northern Fisheries, Stirling, and maintained in freshwater tanks.

Two plaice and two flounders (about 3-years-old and of similar size) were selected, together with two equally sized rainbow trout of approximately 1 year. The fish were killed by a blow on the head, the flatfish within 3 days of capture. Blocks of gill tissue were taken from the middle portion of the gill arches of each species and skin from the upper pigmented and lower non-pigmented surfaces of the plaice and flounder and from the dorsal and ventral surface of the trout. The dissected gill and skin samples were fixed in neutral buffered formalin-saline. Paraffin wax sections were cut at 5 μm and mounted serially.

Sections were stained with one of the following combinations of Alcian Blue–periodic acid–Schiff (AB–PAS): (i) ABpH 2.6-PAS, (ii) sialidase ABpH 2.6-PAS or

(iii) ABpH 1.0-PAS. The details of these techniques, and their assessment (see below), have been described previously (Jones & Reid, 1973*a*,*b*).

With the combined AB–PAS technique goblet cells may stain pure blue (B) or pure red (R) or a combination of these two colours – red with a trace of blue is considered red-blue (RB) and all other combinations as blue-red (BR). In this, as in an earlier study (Jones & Reid, 1973*a*), two varieties of pure blue staining are identified in that a cell may stain either blue or turquoise blue. This variation in staining is distinguished qualitatively but for the purpose of quantification both varieties are counted as pure blue (B).

After ABpH 2.6-PAS those cells staining B and BR are considered to contain predominantly acid glycoprotein and those staining RB or R predominantly neutral glycoprotein. With this staining technique each type of acid glycoprotein may stain with AB (sialomucin sensitive or resistant to sialidase, or sulphomucin). After sialidase ABpH 2.6-PAS only sialidase resistant sialomucin and sulphomucin stain with AB and after ABpH 1.0-PAS only sulphomucin stains. Assessment of the number of cells containing each type of acid glycoprotein is based on a comparison of the population of goblet cells staining with each technique. The difference between techniques (i) and (ii) in the percentage number of cells staining B+BR identifies those cells in which only sialic acid components sensitive to sialidase are available for AB staining: similarly, the difference between techniques(ii) and (iii) in the percentage number of cells staining B+BR identifies those cells in which only sialic acid components resistant to sialidase are available for AB staining. Cells in which only sulphate ester groups are available for AB staining are identified by technique (iii). Where there is an overlap between the techniques in the number of cells staining B+BR, the total number of cells containing only one variety of acid radical cannot be quantified and the possibility of combinations of acid radicals within a cell, each available for AB staining, cannot be excluded (see results).

Quantitative analysis

Goblet cell counts were made under oil immersion (field size 0.18 mm) and the number of goblet cells containing neutral or acid glycoprotein, and each of its types, was expressed as a percentage of the total goblet cell population. Since the difference between species was greater than the variation between individuals of the same species, the results of the two fish of each species were pooled. Similar methods were applied to all three species of fish.

Epidermis

In each section of skin from each fish, 100 goblet cells were characterized by colour after staining with each of the three techniques. The number of cells in each of the four colour categories was expressed per unit length of epithelium (1 mm). In each fish, there was no difference between the distribution of the goblet cells in the pigmented and in the non-pigmented surfaces.

Gill

Fig. 1*a* illustrates the relative positions of the gill secondary lamellae to the gill filament in a teleost gill arch and Fig. 1*b* illustrates the typical branching pattern of the secondary lamellae as seen in a cross-section of gill: goblet cells of the epithelium of a gill filament were counted separately from those of the secondary lamellae.

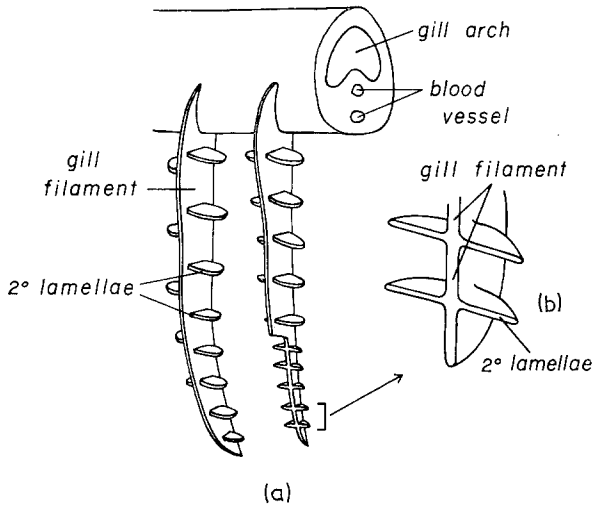

Figure 1. (*a*) The relative positions of the gill filament and secondary lamellae of a teleost gill arch. (*b*) A magnification of the region arrowed showing the typical branching pattern of the filament and lamellae seen in cross-section. Epithelial goblet cells at these two sites were assessed separately.

Gill filament

In each section of gill, 1000 goblet cells were counted from the epithelium of gill filaments selected to include different parts of the middle region of the gill arch. All the goblet cells of each of the selected filaments were counted, and goblet cells of the same filaments were counted in consecutive sections stained by the three techniques. The number of cells in each of the four colour categories was expressed per gill filament and this number then related to 1 mm unit length of epithelium.

Gill secondary lamellae

Similarly, in each section of gill, 1000 goblet cells were counted from the epithelium of the secondary lamellae of selected gill filaments, the same secondary lamellae being counted in consecutive stained sections. The number of cells in each of the four colour categories was counted in each lamella per gill filament and the mean of these values was taken to represent that for 1 mm unit length of epithelium since one lamella represented approximately 1 mm of epithelium.

Results

The number of goblet cells in the epidermis and gill of plaice, flounder and rainbow trout, and their staining characteristics, are shown in Tables 1–3. For each fish species, the percentage number of goblet cells at each site staining B+BR, by each staining technique, is illustrated in Fig. 2. Table 4 shows the percentage number of goblet cells containing acid or neutral glycoprotein.

ANALYSIS OF CELL POPULATIONS CONTAINING SIALOMUCIN

At each site in the flounder and in the gill filament of the rainbow trout the staining response was unusual in that there was an increase rather than a decrease in the number of goblet cells staining B+BR after sialidase ABpH 2.6-PAS as compared with ABpH

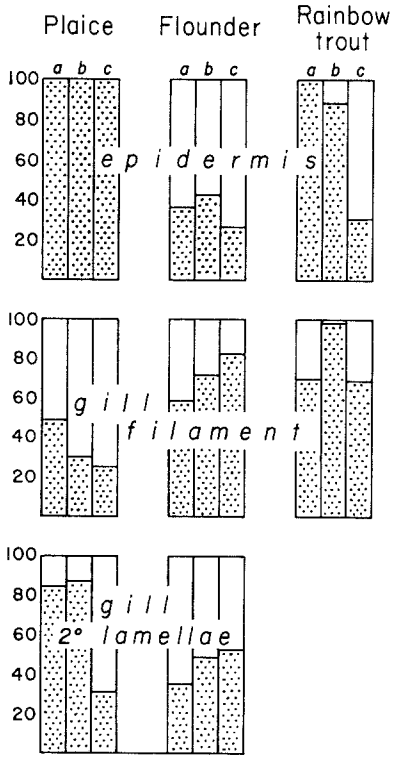

Figure 2. Goblet cells staining blue + blue-red (shaded columns) in the epidermis, gill filament and gill secondary lamellae of plaice, flounder and rainbow trout after ABpH (*a*) 2.6-PAS (sialomucin both sensitive and resistant to sialidase and sulphomucin), (*b*) sialidase ABpH 2.6-PAS (sialomucin resistant to sialidase and sulphomucin) or (*c*) ABpH 1.0-PAS (sulphomucin): glycoproteins demonstrated by each technique given in parenthesis. Results expressed as a % of total goblet cells and calculated from results given in Tables 1–3.

Table 1. Epidermis of plaice, flounder and rainbow trout. Number of goblet cells in 1 mm epithelium and their staining properties: (i) ABpH 2.6-PAS, (ii) sialidase ABpH 2.6-PAS or (iii) ABpH 1.0-PAS.

	Blue	*Blue-red*	*Red-blue*	*Red*	*Total number of cells*
Plaice					
(i)	23.4 (11.30)*	0.2 (0.25)	0	0	23.6 (8.40)
(ii)	22.3 (10.32)	0.1 (0.18)	0	0	22.5 (10.19)
(iii)	23.9 (10.78)	0	0	0	23.9 (1.05)
Flounder					
(i)	0.5 (0.56)	12.5 (12.53)	11.1 (11.06)	11.7 (4.05)	35.9 (19.70)
(ii)	11.3 (11.22)	4.1 (0.71)	10.8 (0.87)	9.4 (7.10)	35.8 (8.58)
(iii)	0.1 (0.20)	9.5 (9.68)	13.6 (13.67)	13.4 (12.10)	36.8 (12.10)
Rainbow Trout					
(i)	41.6 (1.93)	1.3 (1.39)	0	0	43.0 (3.32)
(ii)	13.3 (13.40)	19.7 (16.36)	3.5 (0.67)	0.5 (0.67)	37.2 (2.80)
(iii)	1.5 (1.59)	12.3 (3.07)	23.6 (0.16)	6.8 (4.29)	44.3 (1.78)

* Figures in parentheses are standard errors of the mean.

Table 2. Gill filament of plaice, flounder and rainbow trout. Number of goblet cells in 1 mm epithelium and their staining properties: as for Table 1.

	Blue	*Blue-red*	*Red-blue*	*Red*	*Total number of cells*
Plaice					
(i)	2.1 (0.70)*	12.5 (1.85)	8.0 (1.07)	7.6 (1.85)	30.3 (2.93)
(ii)	2.1 (0.60)	6.5 (1.24)	13.2 (0.88)	6.8 (5.14)	28.7 (2.45)
(iii)	2.6 (0.62)	5.3 (1.35)	17.5 (1.68)	5.7 (1.14)	31.2 (2.69)
Flounder					
(i)	1.5 (0.71)	24.1 (2.94)	13.8 (2.22)	3.9 (0.83)	43.3 (6.13)
(ii)	10.4 (1.29)	17.8 (3.26)	9.5 (2.80)	1.4 (5.54)	39.2 (5.53)
(iii)	2.3 (0.50)	27.4 (3.88)	3.0 (0.89)	3.0 (0.98)	35.8 (7.85)
Rainbow Trout					
(i)	1.3 (0.06)	24.6 (3.47)	8.4 (2.71)	2.6 (1.58)	37.0 (7.46)
(ii)	30.7 (5.02)	5.8 (1.14)	0.5 (0.30)	0	37.0 (5.28)
(iii)	1.2 (0.40)	24.4 (3.50)	9.3 (3.02)	2.0 (0.88)	37.0 (6.53)

*Figures in parentheses are standard errors of the mean.

Table 3. Gill secondary lamellae of plaice, and flounder. Number of goblet cells in 1 mm epithelium and their staining properties: as for Table 1.

	Blue	*Blue-red*	*Red-blue*	*Red*	*Total number of cells*
Plaice					
(i)	0.4 (0.11)*	6.0 (0.55)	1.0 (0.57)	0.1 (0.05)	7.7 (0.60)
(ii)	0.7 (0.26)	4.7 (0.90)	0.6 (0.22)	0.1 (0.07)	6.2 (1.02)
(iii)	0	1.9 (0.04)	2.7 (0.35)	1.6 (0.24)	6.3 (0.75)
Flounder					
(i)	0.2 (0.21)	1.8 (0.40)	3.0 (0.32)	0.6 (0.22)	5.7 (0.70)
(ii)	0.4 (0.22)	2.3 (0.50)	2.7 (0.37)	0.2 (0.20)	5.8 (0.80)
(iii)	0.1 (0.08)	3.5 (0.76)	3.1 (0.64)	0.1 (0.07)	6.9 (1.38)

*Figures in parentheses are standard errors of the mean.

2.6-PAS alone (Fig. 2). This suggests that sialidase removed PAS staining from some cells. Certain cells, previously staining BR, now stained B and since there was an overall increase in the total number of cells staining B or BR, it appeared that some cells previously staining RB had lost PAS staining and shifted to the B+BR category (Tables 1–3). It was striking that those cells now stained B or BR were very pale. This feature of staining precluded identification of goblet cells containing only sialomucin sensitive to sialidase at these sites. In addition, in the gill filament and secondary lamellae of the flounder, the number of goblet cells containing only sialomucin resistant to sialidase

Table 4. Goblet cells (expressed as % of total) containing predominantly Acid (AGP) or Neutral (NGP) Glycoprotein in 1 mm epithelium of epidermis, gill filament and gill secondary lamellae of plaice, flounder and rainbow trout. Based on results of staining with ABpH 2.6-PAS.

	Epidermis		*Gill filament*		*Gill secondary lamellae*	
	AGP	*NGP*	*AGP*	*NGP*	*AGP*	*NGP*
Plaice	100	0	48.3	51.7	84.4	15.6
Flounder	36.3	63.7	59.2	40.8	35.2	64.8
Rainbow Trout	100	0	70.0	30.0	—	—*

*No goblet cells present in rainbow trout secondary lamellae.

could not be assessed since the number of cells staining B+BR after ABpH 1.0-PAS exceeded the number of such cells staining after sialidase ABpH 2.6-PAS (Fig. 2).

EPIDERMIS

The mean epithelial thickness of the skin of the trout was 75 μm compared with 46 μm and 50 μm in the plaice and flounder respectively. More goblet cells were present in 1 mm of epithelium in the rainbow trout (41.5) than in the plaice (23.4) or flounder (36.2) because the epithelium contained more than one layer of goblet cells. In the trout, however, in the thickened epithelium, there was no difference in the staining properties of those goblet cells deep within the epithelium from those at the surface. Only a single layer of goblet cells was present in the skin epithelium of the plaice and of the flounder. In the plaice virtually all goblet cells staining with AB alone stained turquoise blue while in the rainbow trout all such cells stained blue: in the flounder, some cells stained turquoise blue, some blue and some with a patchy mixture of these two colours.

Type of glycoprotein

In both the plaice and rainbow trout all goblet cells of the skin contained acid glycoprotein whereas in the flounder the majority (64%) contained neutral glycoprotein (Table 4).

Of those cells containing acid glycoprotein in the plaice skin, all the goblet cells contained sulphomucin (Fig. 2); these were less frequent in the flounder and in the trout (26% and 31% respectively). In the plaice it may be that with each staining technique some sialomucin in combination with sulphomucin within a cell was masked because AB stained the sulphate radical. In the flounder, cells containing only sialomucin sensitive to sialidase could not be identified since more cells (7%) stained B+BR after sialidase ABpH 2.6-PAS than with ABpH2.6-PAS alone: between 10–17% of goblet cells contained sialomucin resistant to sialidase and some of these acid radicals were available for staining only after the action of sialidase. In the skin of the rainbow trout alone goblet cells containing only sialomucin sensitive to sialidase or sialomucin resistant to sialidase were identified (11% and 58% respectively).

GILL FILAMENT

The mean epithelial thickness of the gill filaments of the three species of fish was similar; plaice 23 μm, flounder 20 μm and trout 17 μm. The mean number of goblet cells per 1 mm of epithelium in the gill filament was 30.1 in the plaice, 39.5 in the flounder and 37.0 in the rainbow trout.

Type of glycoprotein

In each of the three species of fish the goblet cells of the gill filament were identified as containing either acid or neutral glycoprotein. These cell types were present in equal numbers in the gill filament of the plaice but in the flounder and in the rainbow trout aicd glycoprotein containing cells predominated (59% and 70%).

In the plaice gill filament, of those cells containing acid glycoprotein 18% of cells contained only sialomucin sensitive to sialidase, 5% contained only sialomucin resistant to sialidase and 25% contained sulphomucin (Fig. 2). In the gill filament of the flounder and of the rainbow trout no cells containing only sialomucin sensitive to sialidase were identified. In these gill filaments virtually all the goblet cells containing acid glycoprotein included sulphomucin, possibly in combination with sialomucin which here, as in the epidermis of the plaice, would be masked, in each of the techniques, by the AB staining of sulphate radicals. In the flounder and the rainbow trout, however, it appeared that the action of sialidase revealed a further population of cells containing sialomucin resistant to sialidase (13% and 29% respectively) and that in the flounder, since there was a further increase in cell number, some goblet cells contained only sulphomucin staining with AB at pH 1.0 alone (24%).

GILL SECONDARY LAMELLAE

Unlike the other species, in the rainbow trout no goblet cells were present in the epithelium of the gill secondary lamellae. At this site, the mean number of goblet cells per 1 mm of epithelium was 6.8 in the plaice and 6.2 in the flounder.

Type of glycoprotein

In the epithelium of the plaice gill secondary lamellae the majority of goblet cells contained acid glycoprotein (84%) while in the flounder fewer cells did so (35%). Of acid glycoprotein containing cells in the plaice gill secondary lamellae, about 53% contained only sialomucin resistant to sialidase and 31% contained sulphomucin (Fig. 2). In the case of the flounder the pattern of goblet cell staining resembled that seen in the gill filament of this species: all goblet cells containing acid glycoprotein contained sulphomucin, while sialidase revealed more cells containing sialomucin resistant to sialidase alone (14%) and some cells contained only sulphomucin staining with AB at pH 1.0 (17%).

Discussion

The methods of Alcian Blue staining originally developed for the demonstration of epithelial glycoproteins in mammalian tissues have proved suitable, without modification, for the examination of glycoproteins in tissues from both freshwater and marine teleost fish. At the epithelial tissue sites in the three species of fish examined in this study,

the range of glycoproteins was similar to that described at epithelial tissue sites in mammals (McCarthy & Reid, 1964; Lamb & Reid, 1968, 1969; Spicer *et al.*, 1974). Within the fish goblet cell populations, single goblet cells produced either acid or neutral glycoproteins alone or in combination. At some tissue sites, all the goblet cell population produced glycoprotein with acid groups. At other tissue sites the goblet cell population was mixed: some cells produced glycoprotein with acid radicals, some without and some with both. At no site, however, did all the goblet cell population produce glycoprotein without any acid end-groups.

Although quantitative analyses of the types of glycoproteins in fish goblet cells have not previously been made, there is an extensive literature on qualitative aspects of the goblet cell glycoproteins.

Epidermis

Other studies have identified glycoproteins with and without acid radicals in the epidermal goblet cells of teleost fish. Of the glycoproteins with acid radicals, both sialomucins and sulphomucins are widely distributed in teleost epidermal goblet cells (Bremer, 1972). In the eel (Asakawa, 1970), electric fish (Carmignani & Zaccone, 1974) and brown trout (Harris *et al.*, 1973) sialomucins are the major acid component: our finding of the predominance of sialomucins in the epidermis of the rainbow trout is consistent with the finding of Harris *et al.* (1973) in *Salmo trutta*.

The types of glycoproteins in epidermal goblet cells of the adult plaice have not previously been reported and our histochemical findings are of special interest in that all the goblet cell population produced glycoproteins with acid radicals which were sulphated. The presence of sialic acid residues cannot be excluded since histochemical staining of sulphate groups would mask their identification, but an earlier chemical study detected little sialic acid ($<1\%$ on a weight basis) in plaice epidermal secretions (Fletcher, 1968). A neutral glycoprotein fraction has, however, been chemically identified in plaice epidermal mucus, as well as a sulphated fraction (Fletcher & Grant, 1968), although in the present study, no glycoprotein without acid groups was detected. The results of both these studies, taken together, suggest that even at this site a single goblet cell may produce glycoprotein both with and without acid end-groups. It may be that large numbers of sulphate groups staining with Alcian Blue may completely block subsequent periodic acid–Schiff staining of glycoprotein without acid groups i.e. the neutral fraction. A histochemical study by Roberts *et al.* (1973) described glycoprotein without acid groups in mucous cells of larval plaice integument, with the appearance of sulphated material by the 30th day after hatching.

Gill

Histochemical analyses of the glycoproteins of goblet cell populations in the gill filaments of the fish species included in this study have not previously been reported, although detailed accounts of the structure of the gill of the rainbow trout have been published (Morgan & Tovell, 1973; Olson & Fromm, 1973; Morgan, 1974). In an electron microscopy study, Morgan & Tovell (1973) detected few mucous cells and we did not detect any in our light microscope examination.

The predominant type of acid glycoprotein in the goblet cells of branchial epithelium has been found to vary between species (Porcelli & Novelli, 1970; Zaccone, 1972, 1973).

This is consistent with our findings between the fish we examined, although glycoproteins without acid radicals were also present in goblet cells of the gill epithelia. Wright (1974) has reported glycoproteins with and without acid groups in the same mucous cell in the gill of the lungfish, *Lepidosiren paradoxa*.

An increase in the number of cells staining with Alcian Blue after sialidase digestion of the tissue section is unusual e.g., in goblet cells of the gill filament of the flounder and of the rainbow trout and goblet cells of the secondary lamellae of the flounder. This could be explained if the enzyme (Receptor Destroying Enzyme: Wellcome Reagents, Beckenham) removed not only susceptible sialic acid groups but also other sugars, so that resistant sialic acid or sulphate esters were exposed (Jones, 1971). Some fucosidase activity has been established for this enzyme (Das, personal communication). If either resistant sialic acid or sulphate groups were exposed only by the action of sialidase, an increase in Alcian Blue staining at pH 1.0 would not be expected in sections untreated with the enzyme e.g. gill filament of the rainbow trout. The additional increase in the goblet cell population staining with Alcian Blue at pH 1.0 in the gill filament of the flounder could be the result of sulphate staining with Alcian Blue only at this pH level. Such a staining response has been reported at other epithelial mammalian sites (Jones & Reid, 1973*a*).

Comparison of glycoproteins produced within a fish species and between species of fish

The predominant type of glycoprotein produced by epithelial goblet cells varied between tissue sites within a species of fish and between fish species. The greatest difference in the glycoproteins produced at tissue sites within a species occurred in the plaice but the difference between sites in all three fish species contrasted with the uniform staining of the goblet cell glycoprotein in the gill, intestine and epidermis reported in the eel, *Anguilla japonica* (Yamada & Yokote, 1975).

The glycoproteins produced by the epidermal goblet cells showed the greatest interspecies variation. The fish represent species living in marine, freshwater and estuarine environments. In the plaice and rainbow trout all the epidermal goblet cells contained acid glycoprotein although the former were sulphmucins and the latter mainly sialomucins (these fish are representative of marine and freshwater environments respectively). The epidermis of the estuarine flounder contained a more mixed goblet cell population: goblet cells contained glycoproteins both with and without acid groups, the former being either sialomucin or sulphomucin. The flounder, living in a range of salinities, thus showed the greatest range of epidermal goblet cell glycoprotein. By comparison, there was more similarity between the species in the glycoproteins of the goblet cell populations of the gill epithelia.

It is not yet possible to interpret the functional significance of the difference between glycoproteins produced by different goblet cell populations. In mammalian sites a shift in the production of the type of predominant glycoprotein within goblet cell populations has been shown in response to infection (Ventura & Goucher, 1966; Jones *et al.*, 1975) and to irritants (Lamb & Reid, 1968; Jones *et al.*, 1973). The quantitative analysis of the glycoproteins in the epithelia of untreated fish has established a base line which will allow us to compare the synthesis of glycoproteins and to assess any such changes in fish exposed to changed environmental conditions

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