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A histochemical study of the distribution of lectin binding sites in the developing branchial area of the trout *Salmo trutta*

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ABSTRACT

A histochemical study of the branchial area of brown trout embryos from 35 to 71 d of incubation is reported. A battery of 6 different horseradish peroxidase-labelled lectins, the PAS reaction and Alcian blue staining were used to study the distribution of carbohydrate residues in glycoconjugates along the pharyngeal and branchial epithelia. Con A and WGA reacted at every site of the branchial region thus showing the ubiquitous presence of α-D-mannose and *N*-acetyl-D-glucosamine. WGA, DBA and SBA were good markers for the hatching gland cells (HGCs) and mucous cells. Other lectins, such as PNA and UEA I, reacted only for a short time at some sites during the considered period of incubation. From 35 d until posthatching stages, a manifest strong reaction was noted both in the dorsal epithelium of branchial arches and the HGCs as shown by SBA reactivity. This may be significant with regard to the controversial origin of HGCs, which is thought to be endodermal.

Key words: Epithelium; hatching gland cells; glycoconjugates.

INTRODUCTION

In recent years a number of studies have been performed on the characterisation, distribution and significance of glycoconjugates in different organs and tissues of adult teleosts (Zaccone, 1983; Elbal & Agulleiro, 1986*a*, *b*; Witt & Reutter, 1988, 1990; Imagawa et al. 1990; Pastor et al. 1991) including the branchial epithelium (Zaccone, 1972), as well as in larvae (Zaccone et al. 1987). A few studies have been carried out on trout using batteries of labelled lectins (Khan et al. 1991). No studies have been performed on glycoconjugate characterisation in the branchial region either for adult or embryo trout. Most of the studies dealing with the morphogenetic events during the development of the branchial region of trout are descriptive (Knight, 1963; Vernier, 1969; Ballard, 1973*a*, *b*; Ignatieva, 1976) and histological (Vernier, 1976; Morgan & Tovell, 1973; Morgan, 1974*a*, *b*; Kimura & Kudo, 1979). In the present study, the branchial region of trout embryos and newly hatched alevins has been examined histochemically for the first time. Specific sites of interest are the intercellular and apical surfaces of the branchial epithelial cells, the mucous cells and the hatching gland cells (HGCs). The evolution of the saccharidic moieties of glycoconjugates in the developing branchial region of brown trout, which hatches at 65 d, was investigated using horseradish-labelled lectins from 35 to 71 d of incubation.

MATERIALS AND METHODS

Tissue collection, preparation and staining

Fertilised eggs and newly hatched alevins of *Salmo trutta* at different stages of development were collected from a León fish farm (Spain), where eggs were maintained at 10 °C. Groups of 10 eggs were removed from the stock hatchery at days (d) 35, 42, 50, 57, 62, 65, 68 and 71, respectively. After anaesthesia with MS-222, the egg envelope of prehatching embryos was carefully removed by means of sharpened forceps prior to fixation. Denuded embryos were prepared for light microscopy by fixing in 4% paraformaldehyde with 0.25% glutaraldehyde buffered with 0.1 M Sorensen's phosphate buffer at pH 7.0 for 6 h at room

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Table 1. *Lectins and hapten sugars used*

Lectin (acronym)	Nominal specificity*	Concentration (μg/ml) of lectin–HRP conjugate	Hapten sugar**
Canavalia ensiformis (Con A)	αMan > αGlc > GlcNac	10	Man
Triticum vulgare (WGA)	$GlcNAc(\beta1,4GlcNAc)_{1-2}$ > βGlcNAc > Neu5Ac	10	GlcNAc
Dolichos biflorus (DBA)	GalNAcα1,3GalNAc > GalNAc	20	GalNAc
Glycine maxima (SBA)	GalNAcα1,3GalNAc > GalNAc	20	GalNAc
Arachis hypogaea (PNA)	Galβ1,3GalNAc > αβGal	20	Gal
Ulex europaeus (UEAI)	αL-Fuc	20	αL-Fuc

* Carbohydrate specificities of lectins are according to Goldstein & Poretz (1986). ** Concentration of hapten sugars: 0.2 M. Man, mannose; Glc, glucose; GlcNAc, *N*-acetyl-glucosamine; Gal, galactose; Neu5Ac, acetyl-neuraminic acid; GalNAc, *N*-acetyl-galactosamine; Fuc, fucose.

Table 2. *Staining pattern of the branchial region with lectin–HRP conjugates at each stage of development*

		Days of incubation							
Lectin	Branchial region	35	42	50	57	62	65	68	71
DBA	Epithelium	0	0	0	0	0	0	0	0
	Cartilage	—	—	0	0	0	0	0	0
	Mesenchyme	0	0	0	0	0	0	0	0
	HGCs	4	4	3	3	3-2	3-2	—	—
	Mucous cells	—	—	3	3-4	3-4	3-4	4	4
Con A	Epithelium[1]	2-3	2-3	3	3-4	3-4	3-4	3-4	3-4
	Cartilage	—	—	3	3-4	3-4	3-4	0	0
	Mesenchyme	2-3	2-3	3	3-4	3-4	2-3	2-3	2-3
	HGCs	3-4	3-4	4	2	1-2	0-1	—	—
	Mucous cells	—	—	0	0	0	0	0	0
SBA	Epithelium[2]	4	4	4	4	4	4	4	4
	Cartilage	—	—	0	3-4	4	4	0	0
	Mesenchyme	0	0	0	0	0	0	0	0
	HGCs	4	4	2-3	2-3	2	2	—	—
	Mucous cells	—	—	3-4	3-4	3-4	4	4	4
WGA	Epithelium[1,2]	3-4	3	3	3	3	3	3-4	3-4
	Cartilage	—	—	1	1	2-3	3	3	3
	Mesenchyme	3	3	2-3	2	2	2	1-2	1-2
	HGCs	3-4	3-4	4	3	2-3	2-3	—	—
	Mucous cells	—	—	4	4	4	4	4	4
UEA I	Epithelium	0	0	0	0	0	0	0	0
	Cartilage	—	—	0	0	0	0	0	0
	Mesenchyme	0	0	0	0	0	0	0	0
	HGCs	0	0	0	0	0	0	—	—
	Mucous cells[3]	—	—	3-4	3	0	1	2	2-3
PNA	Epithelium	0	0	0	0	0	0	0	0
	Cartilage	—	—	0	0	0	0	0	0
	Mesenchyme	0	0	0	0	0	0	0	0
	HGCs	0	0	0	0	0	0	—	—
	Mucous cells	—	—	0	0	0	3-4	4	4

Intensity of reactivity is shown by symbols: (0) unreactive; (1) traces; (2) weakly reactive; (3) moderately reactive; (4) intensely reactive; (—) structures which are not present. HGCs, hatching gland cells. [1] Cellular membranes; [2] epithelial surface; [3] Mucous cells of epidermis.

temperature. After fixation, specimens were cut into small blocks. Tissue samples were dehydrated through a graded ethanol series and embedded in paraffin. Blocks were serially sectioned (5 μm) and stained with Alcian blue (AB) pH 1.0 and 2.5 and subjected to the periodic acid-Schiff (PAS) and PAS-AB pH 2.5

reactions (Kiernan, 1990). Sections were then examined by light microscopy.

Lectin histochemistry

Tissue sections were treated with 6 lectin-horseradish peroxidase (HRP) conjugates (Table 1) purchased from Sigma (Madrid, Spain). The tissue sections were deparaffinised. Endogenous peroxidase was blocked by a 15 min immersion in 3% H_2O_2 in distilled water. To minimise nonspecific binding reactions, sections were covered for 15 min with 0.1% bovine serum albumin. Following this, they were rinsed in phosphate-buffered saline (pH 7.2) and incubated with a lectin–HRP conjugate for 1 h at room temperature. Each conjugate was diluted with 0.1 M phosphate buffer (pH 7.2). After exposure to the conjugate, the sections were rinsed again, incubated for 8 min in 3-3′-diaminobenzidine-H_2O_2 tetrahydrochloride substrate (Sigma) and counterstained with haematoxylin. Control sections were incubated in parallel for 1 h at room temperature in a mixture of the lectin–HRP conjugate and the appropriate hapten sugar (Table 1) at a concentration of 0.2 M. The hapten sugars were also purchased from Sigma. An additional control was made to check the endogenous peroxidase by using tissue sections which had been reacted with DAB only. Sections were viewed and photographed using an Olympus Vanox H3M microscope.

Assessment of staining

Staining intensity was estimated using the following semiquantitative scale (Table 2): 4, intense stain; 3, moderate stain; 2, weak stain; 1, traces; 0 no detectable reaction.

RESULTS

Morphological and histological observations on the developing branchial area

The sequence of embryonic development in teleosts is continuous: the epithelium covering the gill clefts provides the epithelium of the gill filaments and lamellae. The development of the branchial region, in which the endoderm plays a main role, begins at 6 d of incubation at 10 °C in rainbow trout, and continues until the time of hatching (Morgan, 1974*a*). Immediately after gastrulation the endodermal pharyngeal pouches evaginate ventrolaterally at both sides of the pharynx and after reaching the ectodermal invaginations, they penetrate into the gill clefts (Vernier, 1969, 1976). The development of the pharyngeal pouches to form the 4 branchial arches is completed on d 17 in rainbow trout (Morgan, 1974*a*). The operculum articulates with the hyoid arch to cover the branchial region. The branchial arches are composed of endoderm dorsally and ectoderm laterally; the filaments and lamellae will therefore be composed of ectodermal epithelium (Hamada, 1968; Morgan, 1974*a*). The rudimentary filaments appear in the middle part of the 1st gill arch and then increase in size and number dorsoventrally. The lamellae project from both filament surfaces on d 36 in rainbow trout (Morgan, 1974*b*).

Laurent & Dunel (1978) provided a morphological classification of the gill epithelium: the primary or osmoregulatory epithelium, which covers the branchial arches and filaments, and the secondary or respiratory epithelium, on the lamellae. The former is stratified in several cell layers and intimately related to a venous system. The latter is a bilayered epithelium covering arterioarterial blood compartments. The external layer is composed of specialised pavement cells which bear a labyrinthine pattern of microvilli (Sardet et al. 1979) and the internal layer of undifferentiated cells (Hughes, 1984). There are no mucous cells in the lamellar epithelium but a mucous covering is always present (Lumsden et al. 1994). The gill epithelium differentiates at 28 d of incubation at 10 °C in rainbow trout (Morgan, 1974*b*). At least 5 main types of cells can be distinguished by d 28: pavement, mucous, chloride, hatching gland and undifferentiated. Hatching gland cells differentiate from precursor cells located among endodermal cells a few days after fertilisation (Yamagami, 1988). As development progresses, HGCs migrate caudally to participate in the formation of the foregut. In salmonids, HGCs are distributed on the anterior surface of the embryonic body and yolk sac and on the inner surface of the pharynx and gills. Once the enzyme granules have been released into the buccal cavity and the perivitelline space, HGCs disappear from the epithelia (Yokoya & Ebina, 1976).

AB and PAS histochemistry

The branchial region of the brown trout showed changes in carbohydrate distribution at different developmental stages. The lateral and apical membranes both of the cells of the branchial epithelium and those of the epidermis, together with the basement membrane, showed a strong reaction to PAS up to 50 d (Fig. 1*a*, *b*). In successive developmental stages the reaction became much weaker in the epithelium but increased for the mucous cells, the HGCs and cartilage (Fig. 1*c*). The cartilage of gill arches and

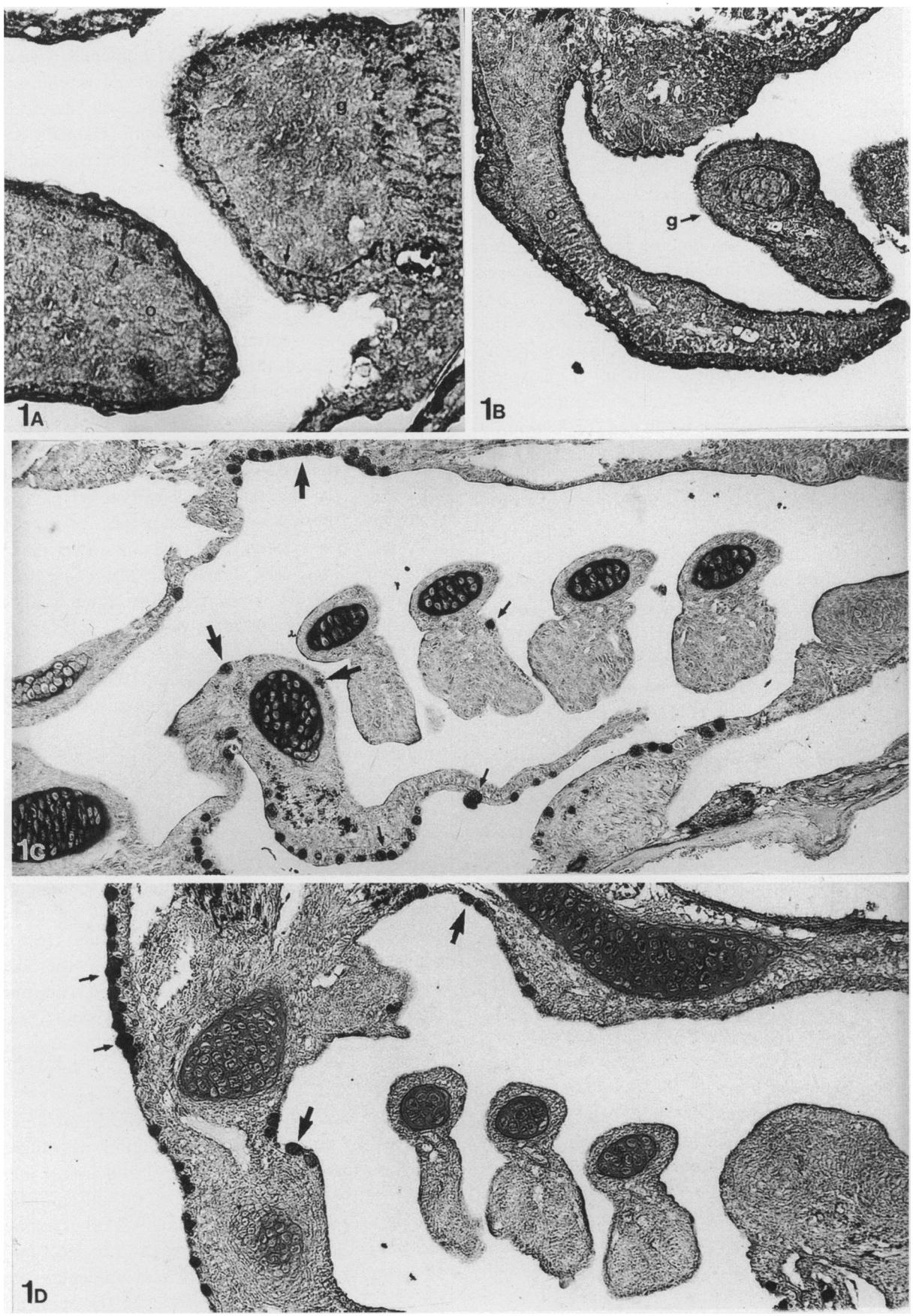


Fig. 1. For legend see opposite.

operculum reacted strongly with PAS as maturation proceeded. HGCs and especially mucous cells showed an intense reaction for PAS from the early stages of development. The staining pattern for AB was similar to that for the PAS reaction, except for the branchial epithelium and the HGCs which did not react with AB. It was thus possible to distinguish between HGCs and mucous cells by the PAS-AB reaction (Fig. 1*d*). The staining intensity at pH 1.0 was always weaker than at pH 2.5 (Fig. 2*a*, *b*). Mucous cells, located in the epidermis of the operculum from 50 d, reacted both with the PAS and AB pH 2.5 stains and the intensity of staining for both reactions increased strongly in successive stages of development (Fig. 2*a*). However, these mucous cells showed a negative to slight reaction with AB at pH 1.0 (Fig. 2*b*). At the hatching stage (65 d) some isolated mucous cells appeared in the gill epithelium of arches and filaments (Fig. 2*c*). Three days after hatching the number of mucous cells in the epithelium of gill arches and pharynx increased (Fig. 2*d*) and it was possible to find a solitary mucous cell in the lamellar epithelium of posthatching alevins (Fig. 2*e*). These branchial mucous cells stained with B at pH 2.5, PAS and AB at pH 1.0 (Fig. 2*f*). The mesenchymal cells and the connective tissue of the gill arches showed a strong reaction with AB pH 2.5. The primordial cartilage of the branchial arches stained both with AB pH 1.0 and 2.5; it was distinguishable by light microscopy from 50 d onwards. The reaction with AB at both pH values in cartilage became stronger at 57 d of incubation, when it showed a positive reaction to PAS.

Lectin histochemistry

WGA

This lectin showed the most intense background staining especially during the final days of incubation that were examined and bound to all components of the branchial region. Both surfaces, that of the branchial region and that of the epidermis, showed a strong affinity to this lectin throughout development (Fig. 3*a*). Neither the basement membrane of the

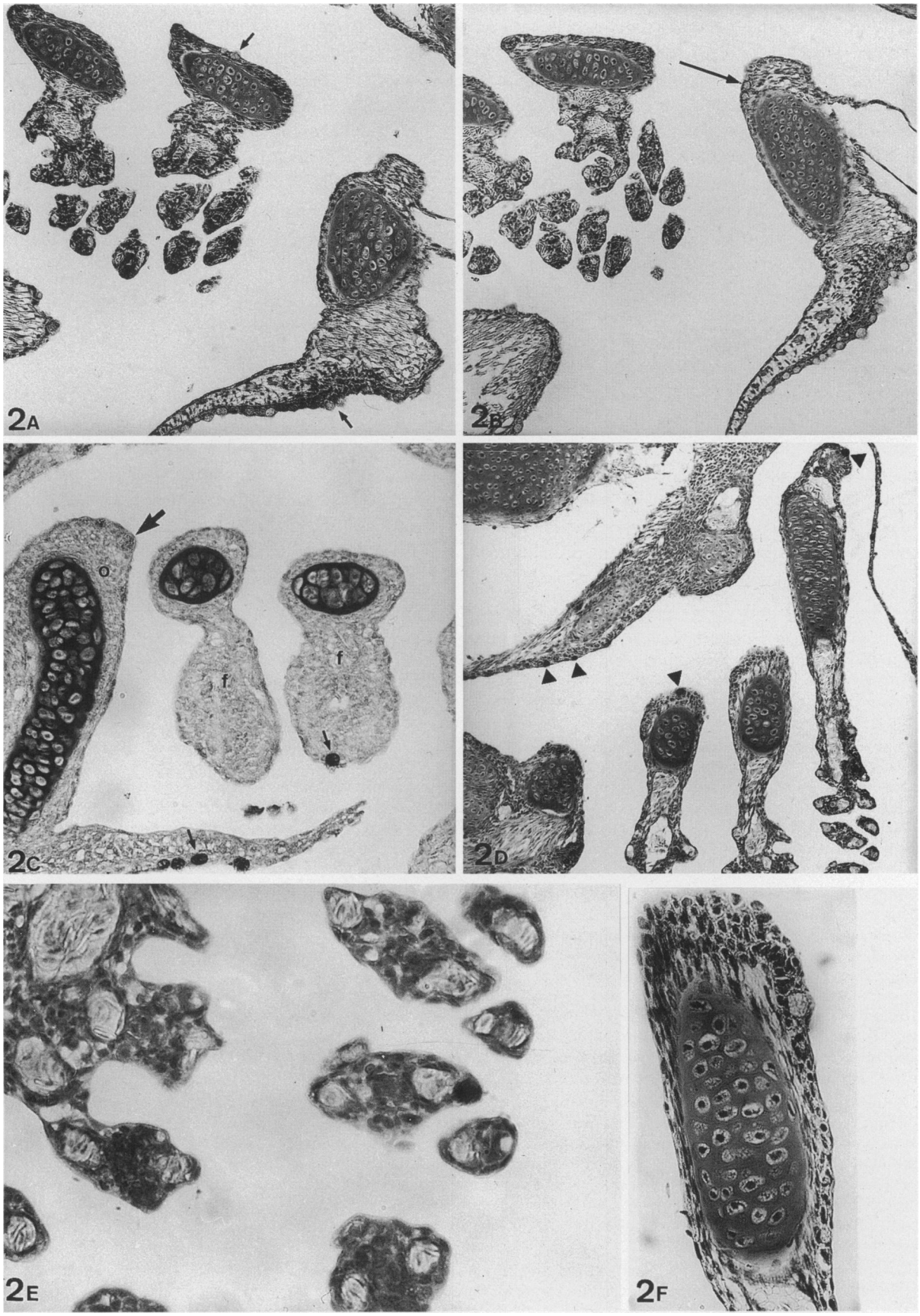
Fig. 1. (*a*, *b*) Transverse sections through the 1st gill arch (g) and the operculum (○) of d 42 and d 50 trout embryos, respectively. Arrows point to the basement membrane of the gill epithelium. PAS; × 1280, × 640 respectively. (*c*) Transverse section of the branchial region of a hatching embryo, stained with PAS. Large arrows, hatching gland cells; small arrows, mucous cells; × 450. (*d*) Branchial region of a hatching embryo stained with PAS-AB pH 2.5. Mucous cells (small arrows) show a stronger staining than hatching gland cells (large arrows); × 500.

Fig. 2. (*a*) Transverse section through the branchial region of a d 68 alevin, stained with AB pH 2.5; all mucous cells (arrows) react with AB pH 2.5; × 700. (*b*) Branchial region of a d 68 alevin stained with AB pH 1.0; only mucous cells in the gill epithelium (long arrow) show significant staining with AB 1.0. × 325. (*c*) Transverse section through the operculum (○) and the 1st and 2nd arches of a d 65 alevin, stained with PAS; f, filaments; small arrows, mucous cells; large arrow, hatching gland cells; × 660. (*d*) Branchial region of a d 68 embryo stained with PAS-AB 2.5. At this stage mucous cells (arrowheads) are numerous; × 660. (*e*) Higher magnification from *d* showing the mucous cells in the gill lamellae of a posthatching embryo; × 1280. (*f*) Gill arch of a d 68 alevin with mucous cells moderately stained with AB pH 1.0; note that the cytoplasm of chondrocytes shows strong staining with AB; × 880.

Fig. 3. (*a*) Transverse section through the hyomandibular arches in a d 50 embryo. Mucous cells (small arrows) show a uniformly strong staining intensity with WGA as compared with the granular staining shown by hatching gland cells (large arrows); ps, pseudobranch; h, hyoid and mandibular (m) arches; × 680. (*b*) Branchial region of a d 57 embryo; hatching gland cells (arrows) show moderate staining with WGA; G, gill arches; ph, pharynx; bc, buccal cavity; H, hyoid arch; × 640. (*c*) WGA staining of the 1st and 2nd gills in a d 62 embryo; hatching gland cells (arrowheads) show weak staining as compared with mucous cells (short arrows). The gill epithelial surface and the cytoplasm of the chondroblast (long arrow) show a moderate staining (3); f, filaments arising from the gill arches; × 700. (*d*) Operculum and 1st gill of a hatching embryo stained with WGA. The different WGA binding sites are located in the mesenchymal cells, blood cells (asterisk), cartilage and the epithelial surface, but the strongest reaction (4) occurs in mucous cells (arrows); arrowheads indicate weakly stained hatching gland cells; × 680. (*e*) In posthatching stages WGA binds to the same structures as before, particularly to the mucous cells (arrows); × 500.

Fig. 4. (*a*) Widespread staining with Con A in the branchial region of a hatching embryo. Only mucous cells (arrows) are unreactive with this lectin; O, operculum; G, gill arches; F, filaments; arrowheads, hatching gland cells weakly stained in the pharyngeal epithelium and yolk sac. × 620. (*b*) Higher magnification from *a* showing the weakly granular staining (1) of hatching gland cells in the pharyngeal epithelium; × 1300. (*c*) Operculum and 1st gill of a posthatching alevin, stained with Con A. Compare this image with Figure 3*e*. Neither the mucous cells (arrowheads) nor the cytoplasm of chondrocytes or the blood cells stain with Con A. Only diffuse staining is observed in the epithelium and connective tissue (arrow); × 840. (*d*) Gills of a d 57 embryo stained with DBA, which binds moderately to hatching gland cells (arrowheads) and strongly to mucous cells in the operculum (arrows); × 640. (*e*) In the gill epithelium of posthatching stages DBA binds only to mucous cells (arrows); × 740.

Fig. 5. (*a*) First and 2nd gills of a d 62 embryo stained with SBA. Compare with Figure 3*c*. Intense staining is shown by the apical cells of the gill epithelium, cartilage, the mucous cells and their secretions; × 660. (*b*) Third gill of a d 57 embryo stained with SBA. Apart from the positive staining of the epithelial surface and cartilage, hatching gland cells (arrow) show moderate staining (2-3); × 1520. (*c*) Gills of a d 71 alevin showing strong staining of the dorsal epithelium of the arches with SBA; F, filaments; L, lamellae; asterisks, branchial artery; × 540. (*d*) SBA staining; operculum of a posthatching alevin with strongly positive mucous cells (arrowheads); × 1200. (*e*) Gills and operculum of a d 71 alevin stained with PNA. Only mucous cells and their secretions (arrows) react with this lectin; × 720.



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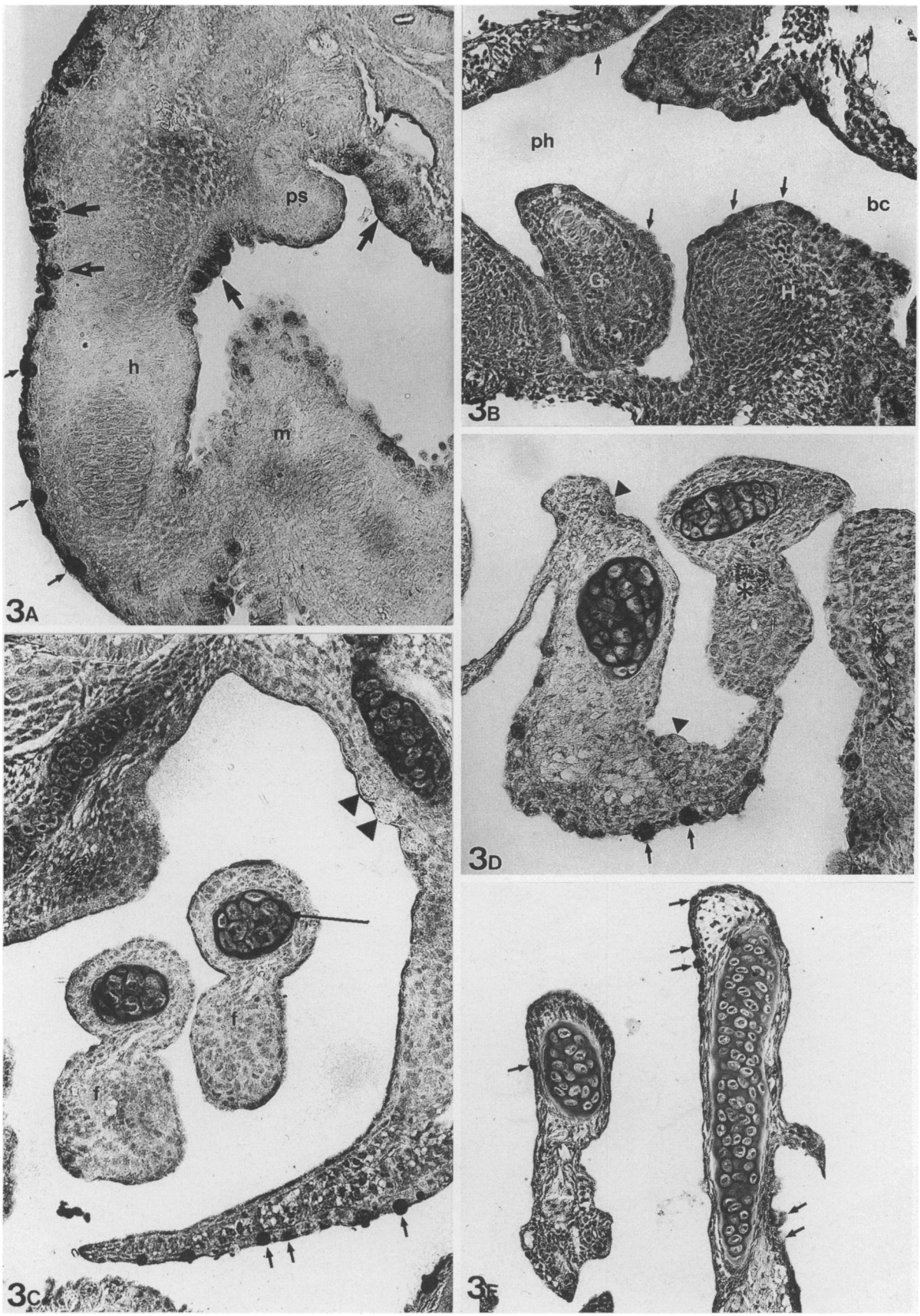

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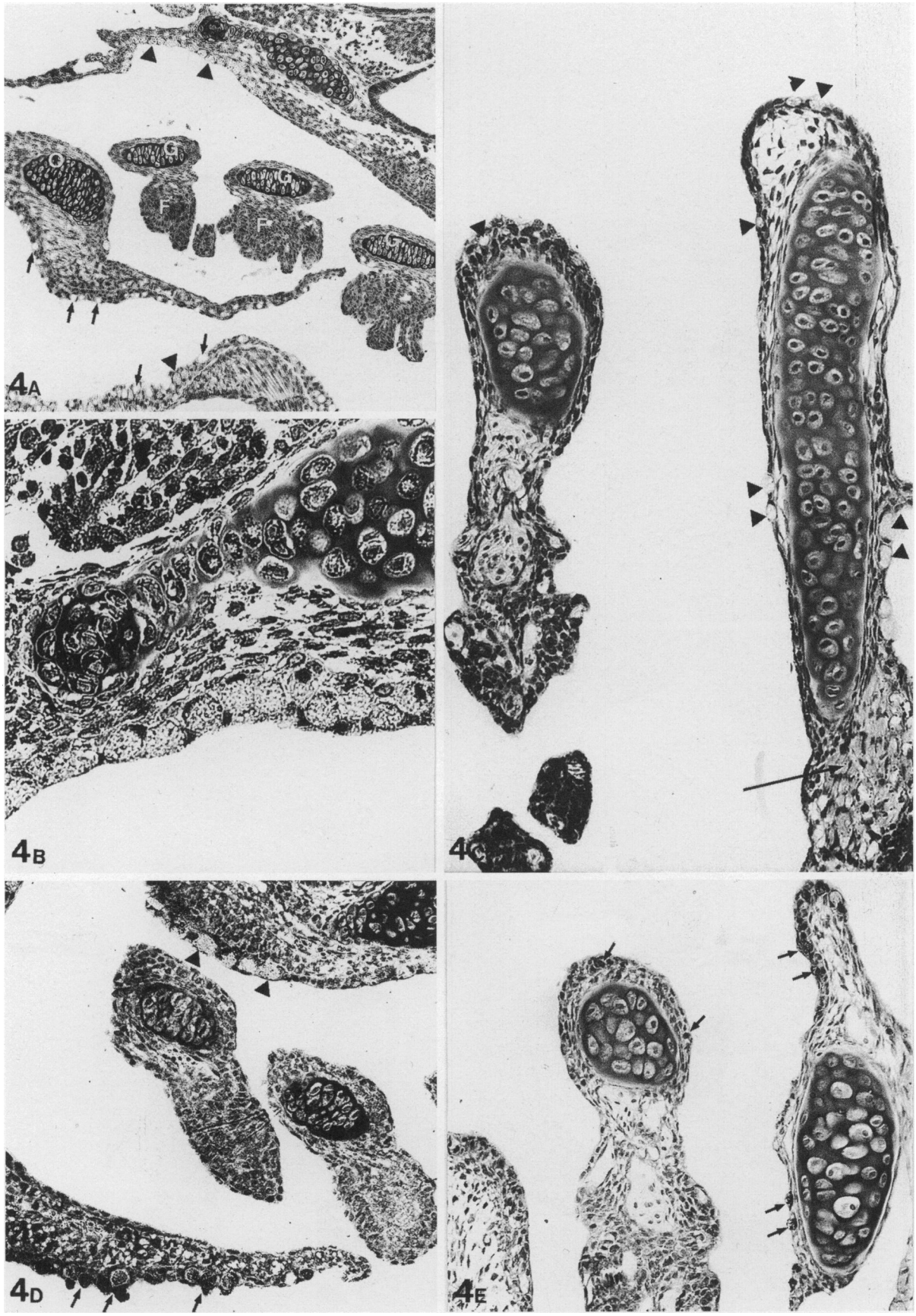

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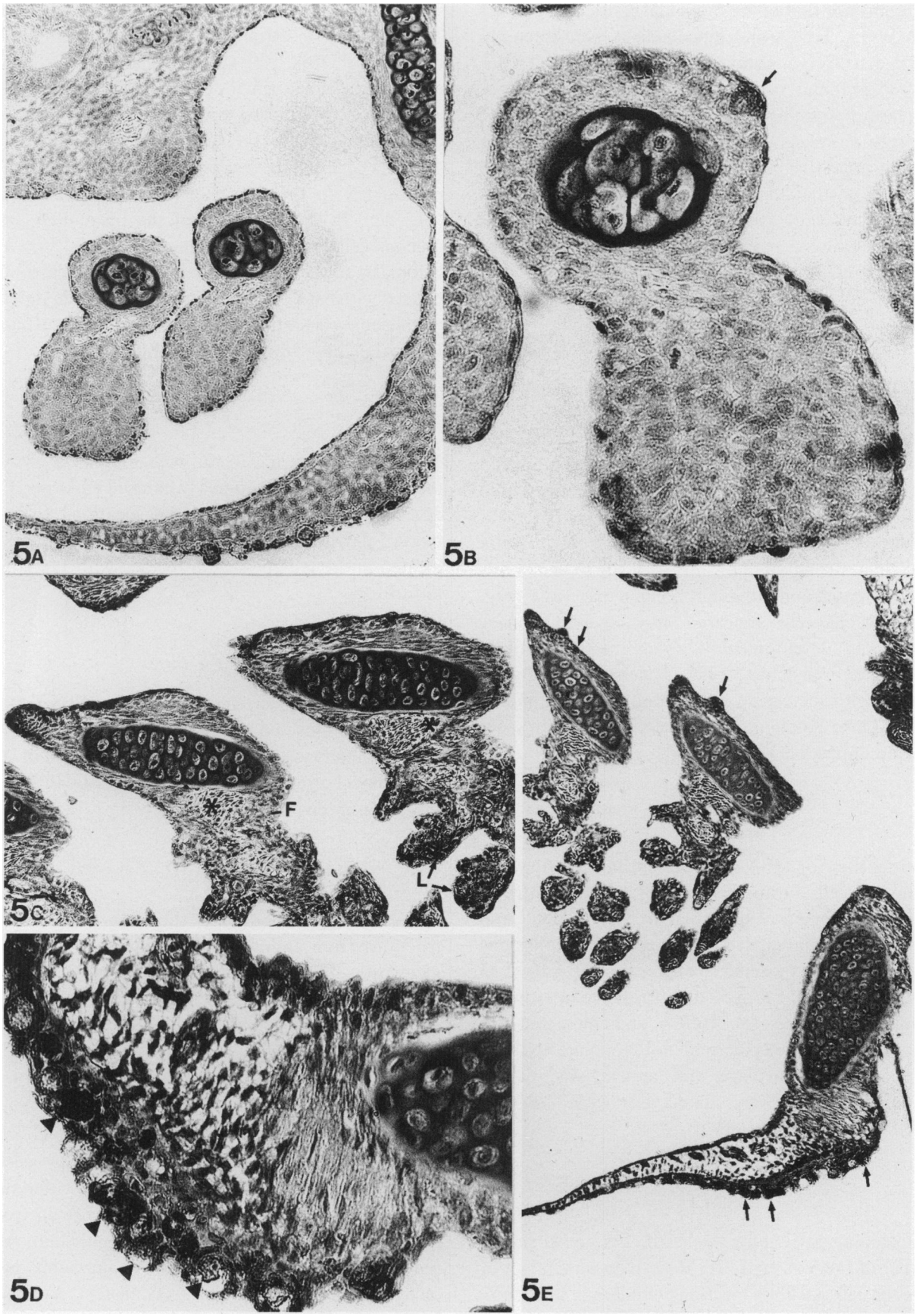

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epidermis nor that of the branchial epithelium stained with WGA. HGCs and mucous cells were intensely reactive to WGA throughout development. HGCs showed granular cytoplasmic staining, and a slight decrease of staining intensity with increasing age of embryos was observed for these cells (Fig. 3*b*, *c*). The staining intensity in mucous cells and cartilage was strong in posthatching stages. The mesenchymal cells and the connective tissue of the gills as well as the blood cells were moderately reactive in all stages studied (Fig. 3*d*, *e*).

Con A

Together with WGA, Con A was the lectin which showed the most intense background, giving rise to widespread staining of the connective tissue and mesenchyme (Fig. 4*a*). In the cells of the branchial epithelium of embryos and alevins, diffuse membrane staining was observed with Con A. The basement membrane did not react with this lectin. HGCs showed a strong staining intensity up to d 57; this then decreased and these cells became unreactive (Fig. 4*b*). Mucous cells appeared unreactive to Con A throughout development. Staining of the cartilage cells of the branchial arches became more intense for Con A with increasing maturation of the chondroblasts. Once hatching took place, the chondrocytes became unreactive (Fig. 4*c*).

DBA

Binding sites for DBA were present in the cytoplasm of mucous cells during the whole of development. The surface epithelium of branchial arches, filaments and pharynx was unreactive. The granular cytoplasmic content of HGCs reacted strongly with DBA from 35 to 57 d (Fig. 4*d*). From 57 d onwards the intensity of staining decreased for HGCs and increased for mucous cells in the epidermal and branchial epithelia (Fig. 4*e*). The cartilage and mesenchyme of the branchial region were unreactive with DBA in embryos as well as in alevins.

SBA

The staining pattern for this lectin was not as similar to that of DBA as expected. Reactivity was shown by mucous cells at both locations, epidermis and branchial arches, by the HGCs and by the gill epithelium (Fig. 5*a*, *b*). HGCs were moderately reactive with SBA up to 57 d. As hatching became closer, the staining intensity for HGCs progressively decreased. This intensity of staining in mucous cells was similar to that for DBA over the same period. The apical membranes of branchial epithelial cells showed high affinity for SBA during embryonic development and this continued into the hatching and posthatching stages. The latter showed prominent staining, located particularly in the dorsal epithelium of gill arches (Fig. 5*c*). In the epidermis of the operculum, only mucous cells showed strong staining (Fig. 5*d*). The cytoplasm of the chondrocytes of the branchial arches reacted with SBA from 57 d onwards and this staining was stronger with increasing maturation of cartilage. In posthatching stages cartilage was unreactive.

PNA

This lectin bound to mucous cells regardless of their location in hatching and newly hatched embryos (Fig. 5*e*). No binding occurred at other locations. In more mature alevins (from 77 d onwards) only mucous cells located in the gill epithelium reacted with PNA (not illustrated).

UEA I

The lectin UEA I, specific for α-L-fucosyl groups of glycocompounds, did not bind in the branchial region of trout embryos. Only the mucous cells of the epidermis showed weak and irregular staining with this lectin in posthatching stages.

Controls

In the control experiments there were no positive reactions from endogenous peroxidase. In the preincubation tests with inhibitory sugars, complete inhibition was achieved for UEA I, PNA, DBA and SBA, and almost complete inhibition for WGA and Con A.

DISCUSSION

The aim of the present study was to identify specific carbohydrate moieties associated with the embryonic differentiation of the gill epithelium in brown trout. Increased staining of the branchial epithelium with time was most obvious for Con A. Since this lectin recognises mannose residues (Goldstein & Hayes, 1978) our study suggests that mannosyl-transferase activity and/or synthesis of endogenous acceptors for mannose increase in the branchial epithelial cells

throughout the developmental period. The slight decrease in the binding of WGA by 42 d of development, followed by an increase after hatching (Table 2) could be the result either of a deposition of new sugars over the binding sites or changes in the steric arrangement of the terminal sugars during the embryonic period from 42 to 65 d. We know from morphological studies that cell differentiation in the epithelium of the branchial arches precedes that in the filaments and lamellar epithelium (Morgan, 1974*b*). Therefore, whatever is responsible for the Con A and WGA staining pattern, it cannot be related to differentiation of the epithelium per se since staining intensity in the gill epithelium was uniform for the arches, filaments and lamellae. Moreover, the increased staining of the gill epithelium with the lectin Con A may merely reflect morphological differences rather than an actual increase in lecting binding to the apical membrane. These morphological differences at the apical surface are due both to the specialisation of pavement cells with time (Sardet et al. 1979; Dunel-Erb & Laurent, 1980) and the secretory activity of mucous cells.

WGA is strongly bound to the epithelial cell surface and to the secretory granules but not to other cytoplasmic components. This pattern was also shown by the PAS reaction. AB staining occurs in the secretory cells and cartilage. At pH 1.0, AB stains only sulphate groups, but at pH 2.5 carboxylated groups (mainly of hyaluronic acid and/or sialic acid) also become stained (Lev & Spicer, 1964). In our results we found that only the mucous cells located in the epithelium of the branchial region stained with AB at pH 1.0. This indicates that these cells contain both sulphated and carboxylated carbohydrates. The strong AB staining at pH 2.5 may also suggest that sialic acid, or even hyaluronic acid, could be components of the surface coat. AB and PAS-AB staining allowed us to identify the presence of solitary mucous cells in the lamellar epithelium, which is at variance with the findings of Laurent (1984) and Lumsden et al. (1994), but in agreement with those of Boyd et al. (1980). The cartilage of the gill arches showed a strong staining intensity with AB pH 2.5 and 1.0 from 57 d, when PAS reactivity became evident. This may be due to the maturation of chondroblasts into chondrocytes. PAS-AB staining was more useful than PAS or AB reactions for distinguishing between HGCs and mucous cells. The PAS and AB reactives bound to the cytoplasmic granules of mucous cells whereas in the HGCs only the PAS reactive showed positive binding.

The sugar specificities of DBA and SBA are similar (Table 1; Schulte et al. 1985). However, in our study, the staining pattern of the branchial epithelium by these 2 lectins during embryonic development was very different (Table 2). Other studies have shown that lectins with similar biochemically defined carbohydrate binding affinities may differ in their staining patterns for the same tissue sections (Schulte & Spicer, 1983; Damjanov, 1987). DBA has been shown to bind only to α-*N*-acetyl-D–galactosamine (Leathem & Atkins, 1983) which does not appear in the gill epithelium during the days of incubation studied. SBA appears to be a specific marker for the epithelium of gill arches and filaments in trout embryos and for the epithelium of the gill arches in alevins, since SBA reacted only in this part of the gill epithelium after hatching. SBA strongly reacted with HGCs regardless of their location. According to Mills & Haworth (1986) the lectin binding to 2 different cell or tissue structures implies a common embryonic origin for them. Although the embryonic origin of HGCs is unclear, from our histochemical results we suggest a common endodermal origin for the epithelium of the gill arches and the HGCs. Yanai (1966) suggested an ectodermal origin for HGCs, but in our study we found that the first appearance of HGCs occurs in the anterior region of the foregut in d 35 embryos and later in the epidermis (d 50 embryos). The epithelial parts of ectodermal origin, such as the epithelium of the lamellae, were unreactive to SBA. This lectin invariably reacted with the epithelium of the gill arches, but the binding of SBA to the epithelium of the filaments occurred throughout embryonic development until some days before hatching. After hatching, no other parts of the branchial epithelium reacted with SBA except for the dorsal epithelium of the arches. This could mean that the filaments have a double embryonic origin, being derived both from ectoderm (Hamada, 1968; Morgan 1974*a*; González et al. 1996) and endoderm. Nevertheless, it seems very likely that the strong staining of the dorsal gill epithelium in alevins can, at least in part, be the result of the deposition of secretion from mucous cells. Apart from the α and α and β- *N*-acetyl-galactosamine residues, the *N*-acetyl-glucosamine and α-D-mannose residues were expressed by HGCs (Table 2). The decreasing of staining intensity in the HGCs as development proceeded was similar for all lectins which reacted with the cytoplasmic granules. This decrease was more obvious when staining with Con A. Therefore, as hatching approaches, the carbohydrate antigens expressed by the HGCs change or are masked, especially the α-D-mannose residues. Since the interpretation of such results is complex further histochemical studies at the ultrastructural level

should be carried out to reach a conclusion regarding the possible endodermal origin of HGCs.

Some lectins such as SBA, Con A and WGA bound to the cartilage cells of the branchial region. WGA bound to cartilage during all stages considered, but SBA and Con A failed to bind to cartilage after hatching. The α and α and β- *N*-acetyl-galactosamine and mannose residues might thus have disappeared because of maturation of the chondrocytes or have been masked.

The fucose binding lectin UEA-I showed no staining reaction in the gill epithelium of the brown trout. It may be that fucose residues are linked as α(1-2) to the terminal galactose of the α-D-galactose-β(1-3)-*N*-acetylgalactosamine sequence and thus may not be freely accessible to the lectin (Khan et al. 1991). Gheri et al. (1990) reported differences in the chick mesonephros in the staining pattern for UEA I and LTA (*Lotus tetranoglobus*) which have the same sugar specificity. It might be expected that staining with LTA would give more information than UEA I in the gill epithelium.

PNA showed a strong reaction in the secretory granules of mucous cells in the gill arch of alevins but did not bind to the same cell type in embryos. In the latter, PNA just bound to the epidermal mucous cells. This observation suggests that as branchial mucous cells mature the carbohydrate antigen to which the lectin binds appears or is unmasked. In more mature alevins (77 d) we found that PNA binds to the branchial mucous cells but not to those on the epidermis (unpublished observation), suggesting the opposite phenomenon mentioned above. Secretory activity in the branchial region of trout embryos starts late in development, when the branchial arches are completely differentiated into filaments. This means that at hatching secretory activity is not fully established since few mucous cells are observed in the gill epithelium. These findings are discrepant from those reported by Morgan (1974*a*, *b*). Pastor et al. (1994) differentiated histochemically between mucous and serous cells. The latter showed high variability in glycoprotein binding to PNA, Con A and WGA lectins. In our results and for the PNA lectin, the staining intensity varied from one secretory cell type to the other, reflecting a complicated heterogeneity of oligosaccharides. The secretory granules of branchial mucous cells contain β-galactose residues, which is in agreement with the studies of Spicer et al. (1983) and Castells et al. (1992) for serous cells. The mucous cells in the gill epithelium of young alevins may be some type of mucoserous cell. Before this hypothesis can be confirmed we must consider other factors which may influence the staining pattern such as the functional status of the secretory cell and the possibility that these mucous cells may constitute a subpopulation (Ito et al. 1991). The progressive establishment of secretory activity is accompanied by a high variability and deposition of glycoconjugates on the cell membrane, but the increase in apical staining intensity may be due to the deposition of mucous secretion from mature mucous cells. In consequence, the staining intensity of the epithelium became stronger with time.

Our study has revealed that glycoconjugates modulate during branchial development in trout embryos. These alterations in the expression of sugars are related not only to the differentiation and cell maturation in the gill epithelium, but also to morphological differences, which complicate the determination of the sugars involved in the development and differentiation of the branchial region. Reaching a clear understanding will require further study of the glycosyltransferases required to form these sugars. The sugar residues which we have detected for longer periods (Table 2) in the early stages of branchial development might be responsible for carrying out the induction and regulation of cellular differentiation processes and subsequently for the regulation of the functional activity of the gills.

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