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Extracellular matrix of connective tissues in the heads of teleosts

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INTRODUCTION

Several types of cartilage can be recognised histologically in teleosts and most are absent from higher vertebrates (Benjamin, 1989*a*, 1990). Briefly, a distinction can be made between cell- and matrix-rich cartilages according to the proportion of cells to matrix (Benjamin, 1989*a*, 1990). Hyaline cartilage can be either matrix- or cell-rich, but all other cartilages are highly cellular. They are Zellknorpel, fibro/cell-rich cartilage, elastic/cell-rich cartilage, hyaline-cell cartilage and scleral cartilage. Zellknorpel supports the gill filaments and has cells that are arranged in columns and separated by thin layers of matrix. Fibro-cell-rich cartilage often forms menisci and elastic-rich cartilage is found in barbels. In routine sections of all these cartilages, the cells are shrunken in lacunae. In hyaline-cell cartilage, the cells have abundant chromophobic cytoplasm and do not shrink. This cartilage is common in the lips of bottom-dwelling cyprinids. Scleral cartilage does not easily fit into cell or matrix-rich categories and is regarded as a special case. It is a thin sheet of cartilage which supports the eye and usually has chondrocytes sandwiched between peripheral layers of matrix.

Tissues which Schaffer (1930) related to cartilage are also found in teleosts. They include mucochondroid, a mucous connective tissue common beneath the skin (Benjamin, 1988), chordoid tissue as represented by the annular ligament of the eye, and the notochord itself. Chondroid bone is also common. It has been regarded as a tissue with properties intermediate between those of cartilage and bone (Huysseune, 1989) and is often seen beneath the surface of synovial joints.

Little is known about the composition of teleostean connective tissues or how their structure is related to function. We describe the distribution of extracellular matrix molecules in a wide variety of teleosts. We show that hyaline cartilage is similar to that in mammals, but that there are striking differences between it and the more cellular cartilages that are absent in higher vertebrates. We also show that teleost bone often contains type II collagen, the characteristic collagen of hyaline cartilage.

MATERIALS AND METHODS

Two fish were examined from each of 12 species of teleosts. The range of species selected ensured that all the cartilages described previously (Benjamin, 1988, 1990) were examined. All teleosts were small and easily identified. The sticklebacks were caught locally, but all other fish were bought commercially.

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The species were the catfishes *Corydoras aeneus* (standard lengths 39 mm) and *Pangasius pangasius* (48 mm, 46 mm) from the order Siluriformes; the Celebes rainbow fish *Telmatherina ladigesi* (32 mm), guppy *Poecilia reticulata* (31 mm, 32 mm) and American flagfish *Jordanella floridae* (28 mm, 36 mm) from the Atheriniformes, the sucking loach *Gyrinocheilus aymonieri* (31 mm), scissor tail *Rasbora trilineata* (28 mm, 38 mm), red eye tetra *Moenkhausia sanctaefilomenae* (26 mm, 28 mm), skunk botia *Botia horae* (30 mm, 32 mm) and coolie loach *Acanthophthalmus semicinctus* (53 mm, 55 mm) from the Cypriniformes; and the 9-spined stickleback *Pungitius pungitius* (22 mm, 25 mm) and 3-spined stickleback *Gasterosteus aculeatus* (37 mm, 41 mm) from the Perciformes.

The fish were killed with an overdose of MS222 (3-aminobenzoic acid ethyl ester; Sigma Chemical Company Ltd, Poole, Dorset, UK). Heads were fixed for 1 h in 90% alcohol, washed in phosphate-buffered saline (0·05 M, pH 7·3; PBS) and decalcified in 5% ethylenediaminetetraacetic acid in PBS for 3–4 days. Previous studies have shown that such decalcification did not affect immunoreactivity with antibodies to chondroitin sulphate, keratan sulphate or type II collagen (Ralphs, Benjamin & Thornett, 1991). After decalcification, material was infiltrated overnight with PBS containing 5% sucrose. The tissue was frozen onto a cryostat chuck using solid carbon dioxide and 10 μm sections were mounted on poly-l-lysine coated slides. Sections were cut from the oromandibular, eye and gill regions. They were labelled by standard procedures for indirect immunofluorescence using monoclonal antibodies to extracellular matrix components (see below). The sections were incubated for 30 min at room temperature with primary antibody diluted in PBS containing 0·1% bovine serum albumin and 0·01% sodium azide. Control sections were incubated with 20 μg/ml nonimmune mouse immunoglobulins or working dilutions of inappropriate monoclonal antibodies. Sections were washed in PBS containing 0·1% Tween 20 and incubated with a 1:80 dilution of fluoroscein-conjugated, rabbit antimouse immunoglobulins (Dako Ltd, High Wycombe, Bucks, UK). After washing as above, sections were mounted in 0·05M tris buffer pH 8·5, containing 33% glycerol, 15% polyvinyl alcohol and 2·5% 1,4 diazobicyclo[2,2,2]octane as fluorescence preservative (after Johnson *et al.* 1982).

Primary antibodies

Type II collagen

Antibody CIIC1 (Holmdahl *et al.* 1986) was obtained from the Developmental Studies Hybridoma Bank maintained by the Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, and the Department of Biology, University of Iowa, Iowa City, IA, under contract NO1-HD-6-2915 from the NICHD. CIIC1 was raised to rat type II collagen and labels hyaline cartilage in other mammals (Holmdahl *et al.* 1986). Its epitope resides in the α1(II) chain. Sections were pretreated with testicular hyaluronidase (Sigma type I-S, 1·45 IU/ml) and chondroitinase ABC (Sigma, 0·25 IU/ml) for 30 min at 37 °C prior to incubation with a 1:2 dilution of culture supernatant (20 μg immunoglobulin/ml).

Keratan sulphate

Antibody MZ15 was a gift from Dr F. Watt (ICRF, London). It was raised to pig chondrocytes and binds to sulphated poly(N-acetyllactosamine) sequences characteristic of keratan sulphate (Zanetti, Ratcliffe & Watt, 1985; Mehmet *et al.* 1986). Sections were pretreated as above and incubated in a 1:1000 dilution of ascitic fluid.

Chondroitin sulphate

Antibody CS56 was obtained from the Sigma Chemical Company Ltd, Poole, Dorset, UK. The antibody was raised to a preparation of chicken gizzard membranes, and binds to the glycosaminoglycan (GAG) moieties of chondroitin 4 and 6 sulphate-containing proteoglycans. It is not cell or species specific (Avnur & Geiger, 1984) and was used at a dilution of 1:200 of ascitic fluid.

Routine histology

Heads were fixed in 10% neutral buffered formol saline, decalcified in 2% nitric acid, wax embedded, sectioned at 8 μm and stained with haematoxylin and eosin or Masson's trichrome (Benjamin, 1990).

RESULTS

Table 1 summarises the distribution of type II collagen, chondroitin sulphate and keratan sulphate in a range of connective tissues. Several of these tissues are particularly characteristic of teleosts and their histological appearance is shown in Figure 1.

Cartilage

Hyaline cartilage. Although 2 forms of hyaline cartilage (cell- and matrix-rich) can be distinguished histologically, there were no differences in immunolabelling characteristics between them. Chondroitin sulphate was present in all fish and was found throughout the matrix. However, labelling was usually strongest pericellularly (Fig. 2*b*). Keratan sulphate was found in 9 species and tended to be restricted to pericellular regions, although it was also seen in the interterritorial matrix in some fish. It was particularly prominent around flattened chondrocytes where cartilage was shortly to be eroded to form a marrow space (Fig. 2*c*). Notably, fish lacking immunolabel for keratan sulphate in hyaline cartilage contained it in cornea. Type II collagen was present throughout the matrix in 11 of the 12 species examined and labelling was usually strong. The fish that lacked type II collagen was *P. pungitius*, although its bone labelled strongly (Fig. 2*d*). In the related stickleback, *G. aculeatus*, label was present but weak in cartilage and was also present in bone.

Scleral cartilage. Scleral cartilage labelled for type II collagen in all species except *P. pungitius*, although labelling was again weak in *G. aculeatus*. Label was strongest in the peripheral acellular zones of most species (Fig. 2*e*). Chondroitin sulphate was generally present throughout the matrix, but keratan sulphate tended to be distributed pericellularly, as in hyaline cartilage (Fig. 2*f, g*).

Zellknorpel. This contained type II collagen in 6 species but labelling was weak (Fig. 3*a*). However, it labelled strongly for chondroitin sulphate and often for keratan sulphate (Fig. 3*b, c*).

Hyaline-cell cartilage. This was seen in the oromandibular region of 3 species and the only molecule detected within it was chondroitin sulphate (Fig. 3 *d*).

Elastic/cell-rich cartilage was present in the barbels of the 2 species of catfish, and contained keratan and chondroitin sulphate, but no type II collagen.

Fibro/cell-rich cartilage was seen in menisci in 3 species. It contained type II collagen in 1 species (in which dense connective tissues were also labelled – see below), but none of the antibodies labelled it in the other 2 teleosts.

Table 1. *A summary of the distribution of chondroitin sulphate* (*CS*56), *keratan sulphate* (*MZ*15) *and type II collagen* (*CIIC*1)

Tissue	Anti-body	Siluriformes		Atheriniformes			Cypriniformes					Perciformes	
Cartilage		*Corydoras aeneus*	*Pangasius pangasius*	*Telmatherina ladigesi*	*Poecilia reticulata*	*Jordanella floridae*	*Gyrinocheilus aymonieri*	*Rasbora trilineata*	*Moenkhausia sanctaefilomenae*	*Botia horae*	*Acanthophthalmus semicinctus*	*Pungitius pungitius*	*Gasterosteus aculeatus*
Hyaline (cell or matrix-rich	CIIC1	+	+	+	+	+	+	+	+	+	+	−	+
	MZ15	+	+	+	+	+	−	−	+	+	−	+	+
	CS56	+	+	+	+	+	+	+	+	+	+	+	+
Zellknorpel	CIIC1	−	−	+	−	+	+	+	+	−	+	−	−
	MZ15	+	+	+	+	+	−	−	+	−	−	+	+
	CS56	+	+	+	+	+	+	+	+	+	+	+	+
Elastic/cell-rich cartilage	CIIC1	−	−										
	MZ15	+	+										
	CS56	+	+										
Fibro/cell-rich cartilage	CIIC1				+			−		−			
	MZ15				−			−		−			
	CS56				−			−		−			
Scleral cartilage	CIIC1		+	+	+	+	+	+	+	+		−	+
	MZ15		+	+	+	+	−	−	+	+		+	+
	CS56		+	+	+	+	+	+	+	+		+	+
Hyaline-cell cartilage (or subtype)	CIIC1						−	−		−			
	MZ15						−	−		−			
	CS56						+	+		+			
Bone	CIIC1	−	+	+	+	−	−	−	−	−	−	+	+
	MZ15	−	−	−	−	−	−	−	+	−	−	−	−
	CS56	+	+	+	+	+	+	+	+	+	+	−	+
Chondroid bone	CIIC1						−			−	+		
	MZ15						−			−	−		
	CS56						+			+	+		
Cornea	CIIC1	−	+	−	+	−	−	−		−		−	+
	MZ15	+	+	+	+	+	+	+		+		+	−
	CS56	−	+	−	−	−	−	+		−		−	−
Ligament	CIIC1	−	+	−	+	−	−	−	−	−	−	−	+
	MZ15	−	−	−	−	−	−	−	−	−	−	−	−
	CS56	−	−	−	−	−	−	−	−	−	−	−	−
Subepithelial connective tissue	CIIC1	−	+	−	+	−	−	−	−	−	−	−	+
	MZ15	−	−	−	−	−	−	−	−	−	−	−	+
	CS56	−	−	−	−	−	−	−	−	−	−	−	+
Mucochondroid	CIIC1		+				−	−			−		
	MZ15		−				−	−			−		
	CS56		−				−	−			−		
Annular ligament	CIIC1			−	−	−	−	−	−			−	−
	MZ15			−	−	−	−	−	−			−	−
	CS56			−	−	−	−	−	−			−	−

Bone

Bone labelled for chondroitin sulphate in 11 species. Label was typically present on the surface, though in some species it occurred in lamellae throughout (Figs 2*b*, 4*a*, *b*).

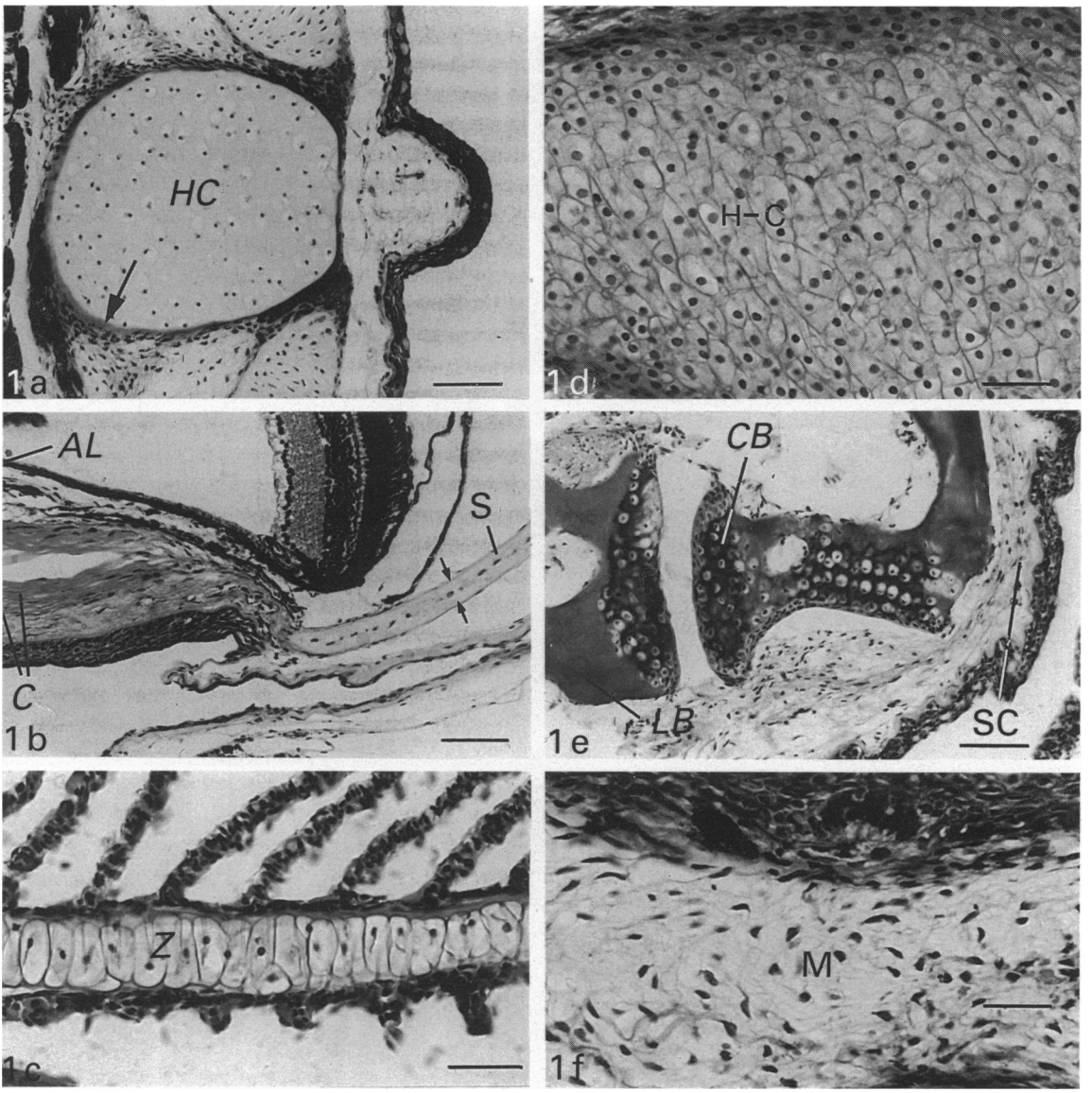

Fig. 1 (*a–f*). Histological sections of selected connective tissues. (*a*) Hyaline cartilage (*HC*) in a gill arch of *Jordanella floridae*. The cartilage is surrounded by a thin layer of perichondral bone (arrow). × 190. Bar = 50 μm. (*b*) The scleral cartilage (*S*) and cornea (*C*) of *Jordanella floridae*. Note the central layer of cells sandwiched between peripheral zones of matrix in the cartilage (arrows). *AL*, annular ligament. × 190. Bar = 50 μm. (*c*) Zellknorpel (*Z*) in the gill filaments of *Gyrinocheilus aymonieri*. × 480. Bar = 20 μm. (*d*) Hyaline-cell cartilage (*H-C*) in the oral sucker of *Gyrinocheilus aymonieri*. × 480. Bar = 20 μm. (*e*) Chondroid (*CB*) and lamellar (*LB*) bone at the articulation of the palatine and the neurocranium in *Acanthophthalmus semicinctus*. Note also the subepithelial connective tissue (*SC*). × 190. Bar = 50 μm. (*f*) Subcutaneous mucochondroid (*M*) in the lips of *Pangasius pangasius*. × 480. Bar = 20 μm. (*a*, *b*, *e*) and (*f*) are stained with Masson's trichrome, (*c*) and (*d*) with haematoxylin and eosin.

In 1 species, bone contained keratan sulphate. Type II collagen was present in the bone of 5 species (Figs 2*d*, 4*c*). Chondroid bone contained chondroitin sulphate in the 3 species in which it was seen (Fig. 4*d*). In *A. semicinctus*, it also contained type II collagen.

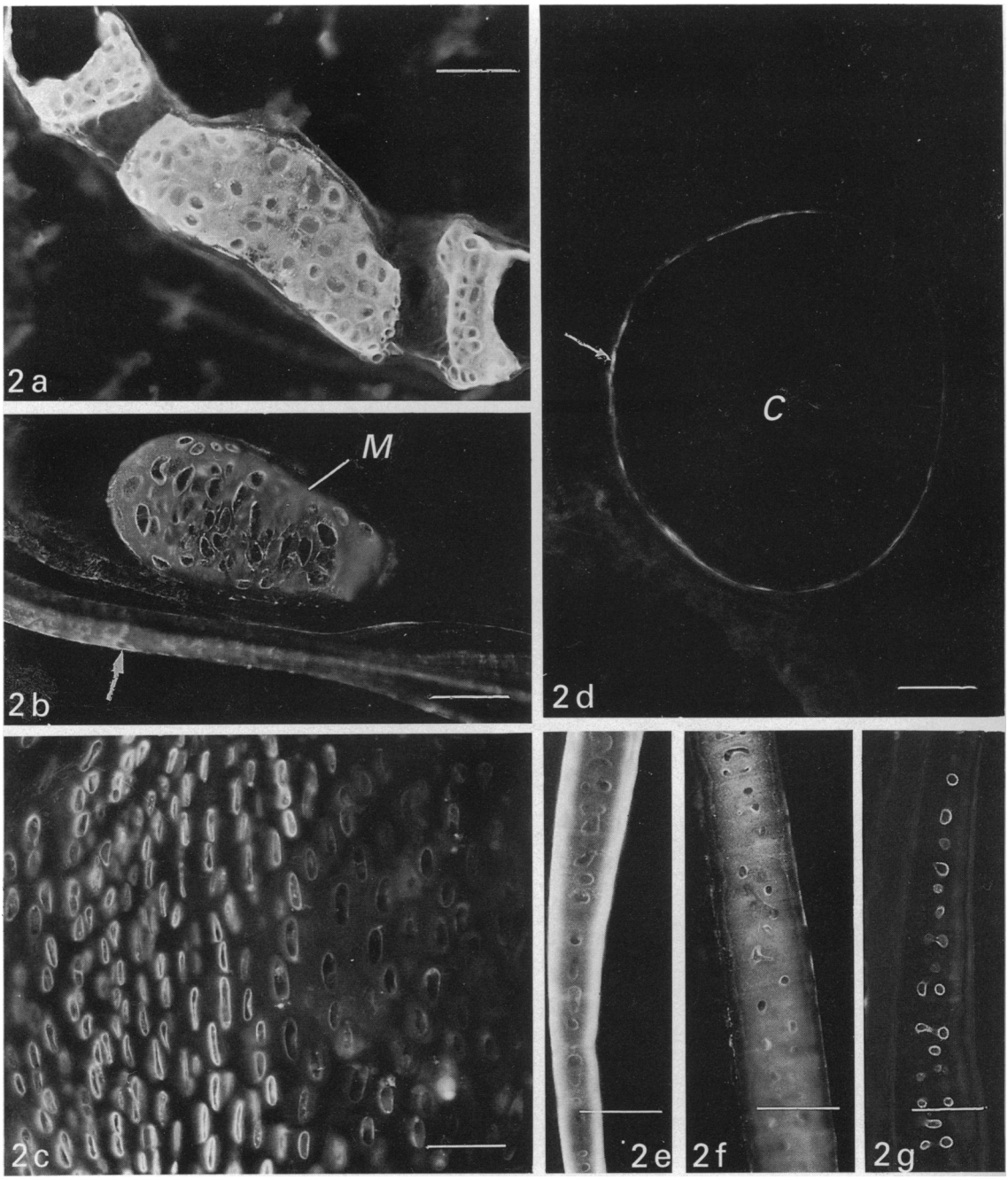

Fig. 2(*a–g*). Extracellular matrix molecules in hyaline cartilage (*a–d*). × 250. Bar = 50 μm. (*a*) Type II collagen in the hyoid arch of *Moenkhausia sanctaefilomenae*. (*b*) Chondroitin sulphate in Meckel's cartilage (*M*) of *Rasbora trilineata*. Immunolabel is distributed throughout the matrix, but is particularly intense pericellularly. Note the chondroitin sulphate in the lamellar bone (arrow). (*c*) Keratan sulphate in the neurocranium of *Jordanella floridae*. The interterritorial matrix is weakly labelled but the pericellular matrix has labelled strongly, particularly around flattened chondrocytes. (*d*) Type II collagen in the hyoid arch of *Pungitius pungitius*. This was the only species where type II collagen could not be detected in cartilage (*C*). However, label was present in the surrounding perichondral bone (arrow, cf. Fig. 1*a*). Extracellular matrix molecules in the scleral cartilage of *Jordanella floridae* (*e–g*). × 250. Bar = 50 μm. (*e*) Type II collagen. (*f*) Chondroitin sulphate. (*g*) Keratan sulphate. The distribution of chondroitin and keratan sulphate was similar to that in hyaline cartilage. Labelling for type II collagen is particularly intense in the peripheral, cell-free zones of matrix.

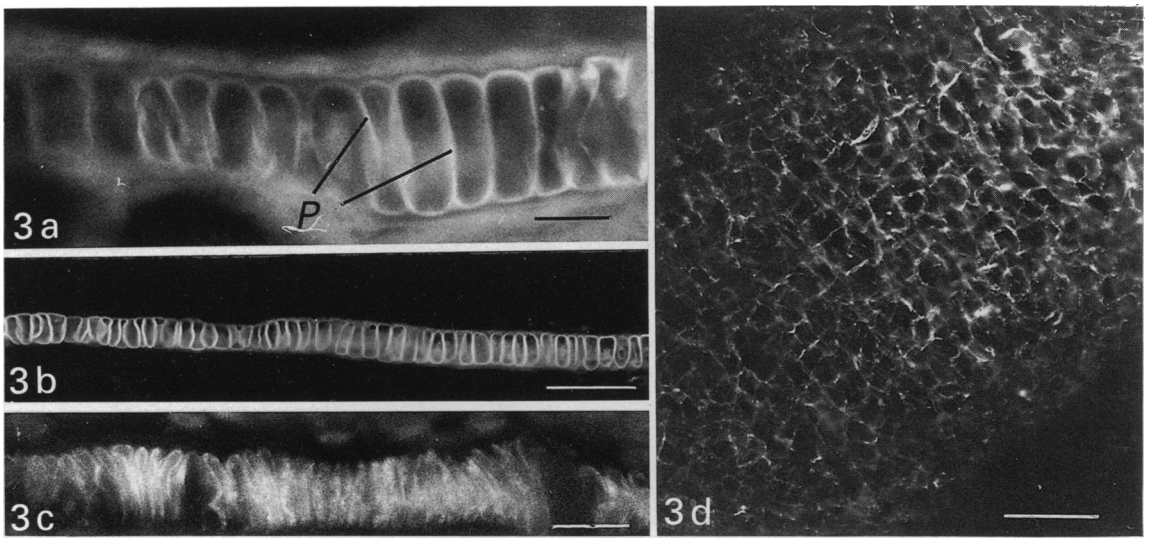

Fig. 3(*a–d*). Extracellular matrix molecules in cell-rich cartilages. Zellknorpel from gill filaments (*a–c*). This cartilage lacks interterritorial matrix (cf. Fig. 1*c*.) (*a*) Type II collagen in *Moenkhausia sanctaefilomenae*. Weak labelling was present pericellularly (*P*). ×900. Bar = 11 μm. (*b*) Chondroitin sulphate in *Gyrinocheilus aymonieri*. ×250. Bar = 50 μm. (*c*) Keratan sulphate in *Jordanella floridae*. ×560. Bar = 18 μm. (*d*) Chondroitin sulphate in hyaline-cell cartilage from the oral sucker of *Gyrinocheilus aymonieri* (cf. Fig. 3*d*) ×250. Bar = 50 μm.

Dense connective tissues

These are cornea, ligament and subepithelial connective tissues (dermis and the lamina propria of the buccal or pharyngeal cavities). Keratan sulphate was present in the cornea of all species (Fig. 4*e*) but was not seen in the other tissues. Chondroitin sulphate was found in the cornea of 2 species but not in ligament or subepithelial connective tissue. Type II collagen was seen in all 3 tissues in 3 species (Fig. 4*f*, *g*). It is noteworthy that these teleosts also contained type II collagen in bone.

Chordoid and chondroid tissues

Mucochondroid contained type II collagen in 1 catfish (Fig. 4*h*), but remained unlabelled by all antibodies in other species. In the notochord of *G. aymonieri*, type II collagen and chondroitin sulphate were present in the notochordal sheath, but the notochord itself was unlabelled. The annular ligament did not label with any antibody in any species.

DISCUSSION

Cartilage

The histological diversity of cranial cartilages in teleosts is accompanied by variation in their matrix macromolecules. The presence of type II collagen in the hyaline and scleral cartilages of 11 species is consistent with its presence in mammalian and avian hyaline cartilage (Mayne & von der Mark, 1983). Type II collagen was not typical of cell-rich cartilages. We did not recognise it in elastic cartilage, although it is found here in mammals (Eyre & Muir, 1975). Type II collagen in hyaline cartilage provides a framework for proteoglycans that confer the space filling and water

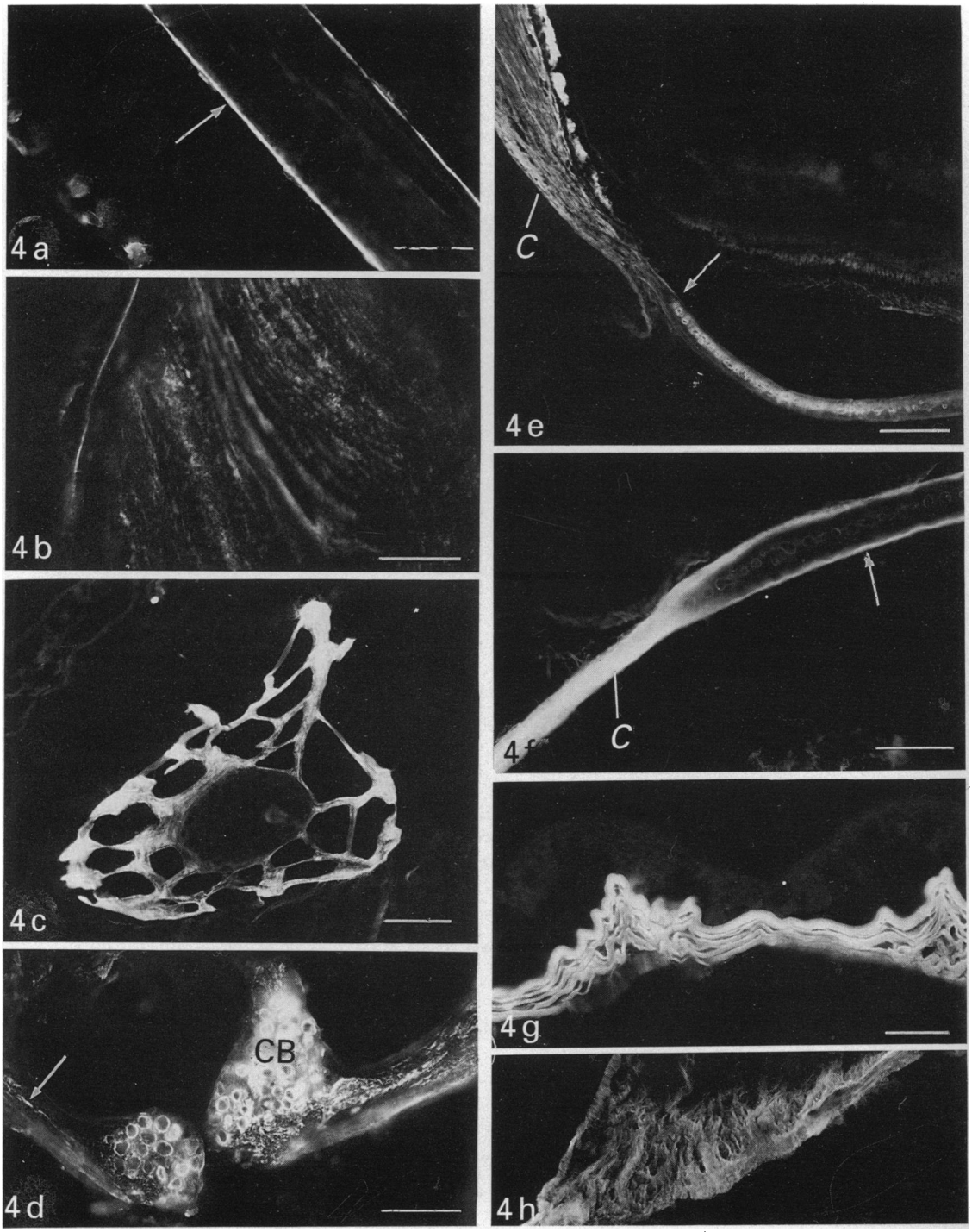

Fig. 4(*a–h*). Extracellular matrix molecules in bone (*a–d*). (*a*) Chondroitin sulphate (arrow) on the surface of a bone of the suborbital series in *Rasbora trilineata*. ×250. Bar = 50 μm. (*b*) Chondroitin sulphate in a bone of the suspensorium of *Botia horae*. Labelling is present within the lamellae. ×250. Bar = 50 μm. (*c*) Type II collagen in cancellous bone from the suspensorium of *Pangasius pangasius*. ×96. Bar = 100 μm. (*d*) Chondroitin sulphate in a joint associated with the neurocranium of *Acanthophthalmus semicinctus*. There is intense labelling in chondroid bone (*CB*) and weaker

trapping properties on the tissue (e.g. Buckwalter *et al.* 1988). Its absence in the most cell-rich cartilages must contribute to their different mechanical properties (see below).

Reasons for the absence of labelling of type II collagen in the cartilage of *P. pungitius* are unclear, especially as its bone labels strongly. It is possible that the epitope recognised by antibody CIIC1 is masked so that it could not be revealed by our enzymatic pretreatment. Alternatively, the epitope could be absent in cartilage collagen of this species, either because of mutational change or posttranslational modifications unique to this tissue. A third possibility is that this species uses a different collagen in cartilage. This last point raises fundamental questions about how different skeletal tissues are defined. Traditionally, hyaline cartilage is defined histologically, although it could now be argued that it be defined by the presence or absence of specific cell products, for example type II collagen. However, the cartilage in *P. pungitius* is structurally and functionally hyaline cartilage just as in the other fish where type II collagen is present. We therefore still think it best to define such cartilages histologically.

Comparing GAG distribution between species, chondroitin sulphate was ubiquitous in cartilages, but keratan sulphate was sometimes absent. Whilst some aspects of differential GAG distribution within a fish are likely to be associated with the tissue-specific arrangement into proteoglycans, there were some clear examples of variation between species. Where a species lacked keratan sulphate in one cartilage it lacked it in all. Species differences in the antigen, leading to nonbinding of antibody MZ15, are unlikely. First, it binds to a specific polysaccharide in keratan sulphate (Mehmet *et al.* 1986). If this sequence is absent then the molecule is not keratan sulphate. Secondly, MZ15 binds to cornea, which is known to be rich in keratan sulphate in mammals and birds (Klyce & Beuerman, 1988). Thirdly, Pfeiler (1991) noted that the amount of keratan sulphate in whole body analyses of marine teleosts varies greatly between species and is absent in some.

Fibro/cell-rich cartilage did not contain either GAG and only labelled for type II collagen in one species (*P. reticulata*). In this fish, type II collagen was present in all dense connective tissues. It seems likely, therefore, that is a dense connective tissue rather than a cartilage.

The cell-rich cartilages have little intercellular material, but the composition of their matrices is diverse. This could be related to their different functions. For example, hyaline-cell cartilage in *G. aymonieri* supports the highly deformable oral sucker that is everted when the fish is attached (Benjamin, 1986); only chondroitin sulphate was detected in the cartilage. Zellknorpel supports the less deformable gill filaments and indeed is often encased in perichondral bone; it has a more complex intracellular matrix. Differences in matrix composition of these cartilages could also be due to their development. Hyaline-cell cartilage can be a secondary cartilage derived from the periosteum of membrane bones (Benjamin, 1989*b*), whereas Zellknorpel is a primary cartilage that is subsequently surrounded by bone.

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labelling in bone (arrow) (cf. Fig. 4*d*). ×250. Bar = 50 μm. Extracellular matrix molecules in dense fibrous connective tissues (*e–g*). (*e*) Keratan sulphate in the cornea (*C*) of *Jordanella floridae*. Arrow, junction between scleral cartilage and cornea (cf. Fig. 1*b*). ×96. Bar = 100 μm. (*f*) Type II collagen in the cornea (*C*) of *Poecilia reticulata*. It is continuous with the dense perichondrial connective tissue of the scleral cartilage (arrow). ×250. Bar = 50 μm. (*g*) Type II collagen in the subepithelial connective tissue of the buccal cavity of *Gasterosteus aculeatus* (cf. Fig. 1*e*). ×96. Bar = 100 μm. (*h*) Type II collagen in subcutaneous mucochondroid of *Pangasius pangasius* (cf. Fig. 1*f*). ×250. Bar = 50 μm.

Bone

Type II collagen is characteristic of mammalian hyaline cartilage and type I is the major collagen of bone. However, our results demonstrate type II collagen in bone of 5 of the 12 species of teleosts examined. It has also been identified in bone of the rosy barb (*Barbus conchonius*) and zebra fish (*Brachydanio rerio*), using an affinity-purified, polyclonal antibody (P. Thorogood, personal communication). It is well known that cartilage is the major skeletal tissue in elasmobranchs. Certain teleosts also rely heavily on cartilage for support, notably the lumpfish *Cyclopterus lumpus* (Davenport & Kjorsvik, 1986). Our results show that cartilage collagen often occurs in teleost bone. It could be that there is a range of bone compositions in teleosts, ranging from highly cartilaginous fish like the lumpfish, through those that contain characteristic cartilage molecules, to fish that do not contain such molecules in bone.

Chondroid bone contained type II collagen in 1 of the 3 species (*A. semicinctus*) in which the tissue was observed. We failed to find type II collagen in the chondroid bone of the others, although it was present in hyaline cartilage. Huysseune (1989) was unable to demonstrate type II collagen in chondroid bone of the cichlid, *Astatotilapia elegans*. She suggested that the cells of this tissue resemble those of cartilage, but its matrix is more like that of bone. Our results suggest that like bone, teleostean chondroid bone shows a spectrum of properties.

Dense connective tissues

A notable finding was the presence of type II collagen in dense connective tissues of *P. pangasius*, *G. aculeatus* and *P. reticulata*. It was striking that this molecule was either in all dense connective tissues or none. In birds and mammals, type II collagen is present in cornea (von der Mark, von der Mark, Timpl & Trelstad, 1977), but its distribution in other dense connective tissues is limited. It is found at the attachment zones of tendons and ligaments (Ralphs *et al.* 1991) and where tendons wrap around bony pulleys (Vogel & Koob, 1989). It may be that the control of type II collagen expression in teleostean dense connective tissues is less specific (i.e. all or nothing) compared with that in birds and mammals.

Chordoid and chondroid tissues

The annular ligament of the eye was regarded by Schaffer (1930) as chordoid tissue, i.e. resembling the notochord in structure. We detected no cartilage molecules in it, but we did find type II collagen in the notochordal sheath of *G. aymonieri*. Type II collagen has been reported here in chick embryos (von der Mark, von der Mark & Gay, 1976). We have not identified the GAGs present in subcutaneous mucochondroid, although the space-filling nature of the tissue suggests that hyaluronic acid may be important. Type II collagen was found in the mucochondroid of *P. pangasius*, but not in the other 3 species in which the tissue was observed. As *P. pangasius* was the only one of these 4 species where type II collagen was present in dense connective tissue, the collagen type in mucochondroid may be regulated in the same way as that in dense connective tissues.

SUMMARY

The distribution of extracellular matrix molecules (chondroitin and keratan sulphates, type II collagen) is described in cranial connective tissues of teleosts. Hyaline cartilage was similar to that in mammals and usually contained all 3

molecules. The more cellular cartilages that are not normally present in mammals were more variable in composition. Scleral cartilage closely resembled hyaline cartilage, Zellknorpel in the gill filaments resembled it in some species but not in others, and elastic/cell-rich and hyaline-cell cartilages were unlike hyaline cartilage. These variations may be related to functional or developmental differences between the tissues. Bone and chondroid bone also varied in composition between species. Whilst both tissues contained chondroitin sulphate, bone contained type II collagen in 5 of the 12 species examined. This suggests that cartilage components are more widespread in teleost bone than has previously been shown. Type II collagen also occurred in dense connective tissues of some species. Notably, where this molecule was present in one of these tissues, it was present in all.

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