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A comparative study of lectin binding to cultured chick sternal chondrocytes and intact chick sternum

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Cultured chondrocytes derived from the caudal and cephalic ends of embryonic chick sterna have been compared with each other and with whole sternum, by using a panel of 21 lectins to probe the distribution of oligosaccharides in glycoconjugates of cells and matrix at various times of culture or development. On culture in collagen gels, the cells changed their morphology with time, degrading glycan in the surrounding culture medium and depositing new matrix, the glycan content of which reflected the site of origin of the cells, indicating that the glycan phenotype of both cells and matrix ('glycotype') was predetermined and persistent. Sterna of embryonic chicks showed unexpected complexity in their distribution pattern of glycan, containing at least six distinct regions. Major regional temporal differences were evident among saccharides terminating in *α*-*N*-acetyl galactosamine and *β*-galactose, while changes in glycans terminating in fucose, sialic acid and *α*-mannose were somewhat less marked. Subsets of complex N-glycans changed little.

***Keywords*: lectin binding; chick sternum; chondrocyte culture**

Introduction

It has long been known that there are two types of cartilage in the embryonic chick [1]. At 17 days *in ovo*, the chick sternum consists of hypertrophic cartilage containing large chondrocytes at its cephalic end and small-celled cartilage, which does not calcify, at its caudal end [2]. Chondrocytes from chick embryos have been grown in collagen gels [2–9] and differences have been found in both morphology and metabolic activity between those derived from the cephalic and caudal portions of the sternum. Initially matrix-free chondrocytes of both types showed a spindle-shaped morphology at 1–2 days in culture, becoming rounded by 5–6 days, by which time they had produced cartilage-like nodules of matrix [4]. Cells of cephalic origin were vacuolated and were larger than their caudal counterparts, but produced smaller nodules [6, 8]. Synthesis of type X collagen was restricted to the cephalic cells [2].

The various zones of articular cartilage differ in their lectin-binding characteristics *in situ* [10–12] and chondrocytes of the superficial and deep zones produce different proteoglycans on culture *in vitro*, both in man [13] and domestic cattle [14]. The study described here was undertaken to determine whether there were similar regional differences in the glycans of the matrix and chondrocytes of chick sternum and whether any such variations persisted in tissue culture.

Materials and method

Sterna from 6-week-old chickens were fixed in 10%(v/v) neutral buffered formalin. Blocks were taken at the junction of cartilage and bone, which were decalcified in a saturated, aqueous solution of ethylenediamine-tetraacetic acid, pH 7.4 (BDH Chemicals Ltd, UK). Sterna from 17 day *in ovo* chick embryos were fixed in 2.5%(v/v) glutaraldehyde (Agar Scientific Ltd, UK) in 0.1 M sodium cacodylate, pH 7.1 (Bio-Rad Laboratories Inc., UK).

Cell culture

Chondrocytes were isolated from the caudal and cephalic ends of the sterna of 17 day *in ovo* chick embryos using the method of Gibson *et al.* [6]. Sterna were incubated at 37 °C with 1 mg ml^{-1} collagenase (type I crude, Sigma Chemical Co. Ltd, UK) and 0.03%(w/v) trypsin (Life Technologies Ltd, UK) in Dulbecco's modified Eagle's medium (DMEM, Life Technologies Ltd) for 30 min, washed and divided into three portions. The caudal and cephalic portions were separately pooled, minced with a scalpel and incubated with fresh enzyme solution for 90 min. The released, matrix-free chondrocytes were washed three times with complete medium (DMEM containing penicillin 100 U ml^{-1}, streptomycin 100 μg ml^{-1}, L-glutamine 2 mM (all from Life

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Technologies Ltd), 25 μg ml^{-1} L-ascorbic acid (Sigma Chemical Co. Ltd) and 10%(v/v) foetal calf serum (Life Technologies Ltd), counted, mixed with DMEM in 2 mg ml^{-1} collagen (type I extracted from rat tail tendons) at 1×10^5 cells ml^{-1}, and 2 ml of this mixture added to 35 mm culture dishes (Falcon, non-tissue culture treated, Becton Dickenson Ltd, UK). The cells were fed after the mixture had gelled, and then every 2–3 days, with a 2 ml layer of complete medium.

'Caudal' cell cultures were fixed on days 2, 3 and 4, and both 'caudal' and 'cephalic' cell cultures were fixed at day 7 and day 21 in 2.5%(v/v) glutaraldehyde in 0.1 M sodium cacodylate. The fixed sterna and gels were processed to paraffin wax and 5 μm sections cut. The sections were treated with a panel of 21 lectins (see Table 1 for source and specificities) using the avidin-biotin complex method of Jones *et al.* [15], but using 0.03%(w/v) trypsin (type II crude, Sigma Chemical Co. Ltd, UK), which was found to be the optimum concentration for these tissues in preliminary experiments, instead of 0.1%(w/v) trypsin for pretreatment of the sections. The biotinylated lectins DSA, GNA, MAA and SNA were obtained from Boehringer Mannheim Ltd, UK, STA from Vector Laboratories, UK, and the remainder from the Sigma Chemical Co. Ltd, UK. Control sections, treated with diluent buffer instead of lectins, were included with every staining run. Lectins were tested for specificity by using blocking sugars at 0.2 M mixed with the lectins before use. LCA and PSA were blocked with mannose, STA and DSA with *N*-acetyl glucosamine, ECA and CTA with galactose, and HPA, WFA and SBA with *N*-acetyl galactosamine. SBA was also incubated in the presence of 0.2 M methyl α-D-mannopyranoside. Some sections were treated with enzymes after the trypsin pretreatment and before lectin staining. Neuraminidase (type VI, from *Clostridium perfringens*) at 0.1 U ml^{-1} (in 0.2 M acetate buffer, pH 5.5, containing 1%(w/v) calcium chloride) was added for 1 h at 37 °C to the sections, washed and stained with SNA and MAA. α-Mannosidase (from almonds) at 1 U ml^{-1} in 0.1 M citrate buffer, pH 4.5, was added for 1 h at 26 °C (4 changes of 15 min each) prior to staining with the lectins GNA, ePHA and lPHA. The α-mannosidase solutions were checked for residual activity by reaction with *p*-nitrophenyl α-D-mannopyranoside. α-L-Fucosidase (from bovine epididymis) at 0.25 U ml^{-1} (in 0.1 M citrate buffer pH 6.5 containing a trace of bovine albumin) was added for 2 h at 26 °C prior to treatment with UEA-1. All enzymes and sugars were from the Sigma Chemical Co. Ltd, UK

Results

The results of lectin binding were assessed using a ranked scoring method in which 0 represents none, 1 weak, 2 moderate, 3 strong and 4 very strong, intense staining.

Table 1. Lectins used in this study.

Acronym	*Source*	*Major specificity*
GNA	*Galanthus nivalis*	Manα1,3Manα1- [27]
PSA	*Pisum sativum*	non-bisected bi/tri antennary N-glycan [29, 49]
LCA	*Lens culinaris*	non-bisected bi/tri antennary N-glycan [49]
ePHA	*Phaseolus vulgaris*	bisected bi/tri antennary N-glycan [28,17]
lPHA	*Phaseolus vulgaris*	non-bisected tri/tetra antennary N-glycan: influenced in some cases by position of terminal residues [28]
STA	*Solanum tuberosum*	Galβ1,4GlcNAcβ1-/GlcNAcβ1,4GlcNAc- [50,51] ↓ increasing affinity for multiple branches of repeated NAcLac
LEA	*Lycopersicon esculentum*	similar to STA [52]
DSA	*Datura stramonium*	similar to STA [31, 53]
AHA(PNA)	*Arachis hypogaea*	Galβ1,3GalNAcα1- $\gg$ Galβ1,4GlcNAcβ1- $>$ Galβ1,3GlcNAcβ1- [41, 42]
ECA	*Erythrina cristagalli*	Galβ1,4GlcNAcβ1- $>$ Galα1,3Galβ1,4GlcNAc- [32, 35]
CTA	*Erythrina corallodendron*	Galβ1,4GlcNAc-, especially in multiple termini [32, 54]
HPA	*Helix pomatia*	GalNAcα1- [37, 38]
WFA	*Wisteria floribunda*	GalNAcα1,6Galβ1- $>$ GalNAcα1,3Galβ1- [37, 55]
SBA	*Glycine max*	GalNAcα1,3Galβ1-, in longer glycans [34, 56]
MPA	*Maclura pomifera*	Galβ1,3GalNAcα1- and GalNAcα1- $>$ Galα1- [39, 40]
DBA	*Dolichos biflorus*	GalNAcα1,3(Fucα1,2)Galβ1,3/4GlcNAcβ1- (blood group A-like) [36, 57]
UEA-1	*Ulex europaeus*	Fucα1,2Galβ1,4GlcNAcβ1- (blood group O; 'H' antigen) [58, 59]
LTA	*Tetragonolobus purpureus*	certain Fucα1- in clusters, -4GlcNAcβ1,4(Fucα1,6)GlcNAcβ1- [60]
BSA-1B4	*Griffonia simplicifolia*	Galα1- (blood group B-like)/Galα1,3 $\pm$ (Fucα1,2)Galβ1,4GlcNAcβ1- [61, 62]
MAA	*Maackia amurensis*	NeuNAcα2,3Gal- [63, 64]
SNA	*Sambucus nigra*	NeuNAcα2,6Gal/GalNAc- $>$ Galβ1,4GlcNAc- [65, 66]

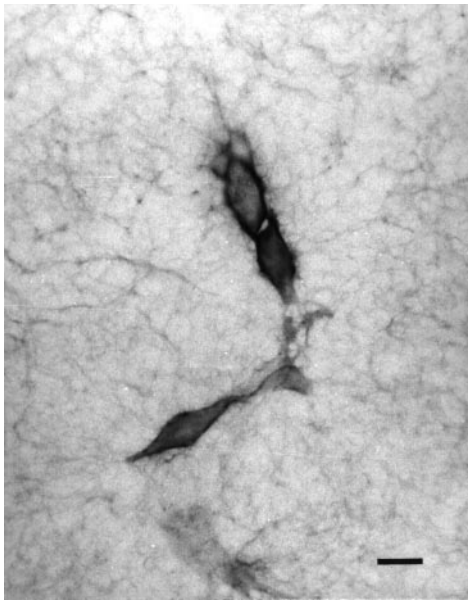

Figure 1. Chick sternal chondrocytes cultured for 3 days in collagen gels and stained with the lectin WFA illustrating staining of the surface of both spindle shaped and round cells. Bar = 10 μm.

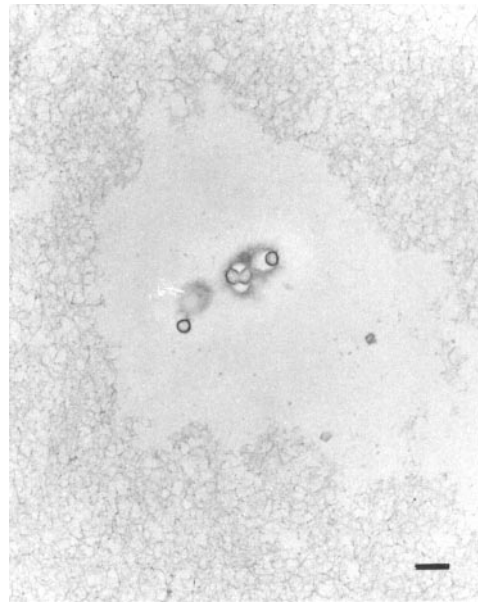

Figure 2. Chondrocytes from the caudal end of the chick sternum cultured in collagen gel for 7 days and stained with the lectin LCA. There is a halo around the cells where there is weaker staining, the area directly around the cells being least stained. Bar = 20 μm.

Cultures of 'caudal' cells were examined at 2, 3 and 4 days to compare binding of the lectins to spindle-shaped cells and to rounded cells (Figure 1). GNA bound to the round cells by day 4, but did not bind to the spindle-shaped cells. MPA, AHA, ECA, CTA and WFA stained the round cells slightly more strongly than the spindle-shaped cells. LPHA did not bind to any cells on day 2, but gave weak staining of cells of both shapes on days 3 and 4. UEA-1, DBA, BSA-1B4, HPA and SBA were totally negative in these cultures. None of the lectins bound to spindle-shaped cells alone.

Tables 2 and 3 summarize the results of the binding studies on the 7 and 21 day cultures, and the embryonic sternum. Overall, at 21 days, the matrices of the 'caudal' cultures (not including the peri-lacunar matrix) bound lectins more intensely than those of the 'cephalic' cultures. It was possible to distinguish different patterns of staining within the matrix of the nodules, apart from the peri-lacunar region. Some lectins (*eg* LTA) tended to stain evenly throughout the nodular matrices, whereas others (*eg* SNA) stained mostly at the outer edges of the nodules. There was a low level of background staining of the collagen gel with most of the lectins. With many of the lectins, especially with WFA, STA, SNA and LCA, a distinct 'halo' of unstained gel was observed around the nodules from 2 to 3 days in culture (Figure 2).

The lectin binding studies performed on the 17 day old chick embryo sternum demonstrated that there were at least six regions in the sternum distinguishable by their patterns of lectin binding. These regions are indicated in Figure 3. The more mature sternum of the 6-week-old chickens also demonstrated differences in the lectin staining patterns of

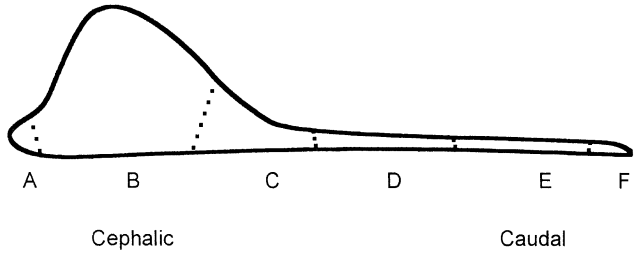

Figure 3. Lateral scheme of the embryonic chick sternum showing the six regions which are distinguishable by their patterns of lectin binding.

adjacent areas of cartilage (Figure 4). The binding to the mature cartilage nearest to the calcifying region was similar, for most lectins, to that found in regions B and C of the embryonic sternum, although ECA and CTA bound to cells of mature sternum, but not to those of the embryo, whereas BSA-1B4 bound to embryonic, but not to mature sternum.

Figure 5 illustrates binding of WFA to the six regions of the sternum. All regions showed strong staining, with greatest intensity in the area between the rows of cells (*ie* the interterritorial matrix) which was especially distinct in regions E and F. Figure 6 shows binding by STA which stained the territorial matrix more strongly than the interterritorial matrix. In the mature sternum, by contrast, STA showed selectivity for interterritorial matrix while WFA, in one area only, had most binding in the territorial matrix (Figure 4). GNA bound only to intracellular granules.

Table 2. Staining of cultured chick sternal chondrocytes and chick embryonic sternum by lectins that bind to internal glycans. Key to areas: CELL, intracellular staining; CELL SURFACE, staining of the surface of the cells; PERI-LACUNAR, staining of the matrix immediately outside the lacunae of the chondrocytes; MATRIX, staining to the matrix excluding the peri-lacunar region. G, granular, intracytoplasmic staining. 0 represents no staining, 1 weak, 2 moderate, 3 strong and 4 very strong, intense staining.

Lectin	*Area*	*Cultured sternal chondrocytes*				*Embryonic sternum (regions)*					
		'Cephalic'		*'Caudal'*		*A*	*B*	*C*	*D*	*E*	*F*
		7 day	*21 day*	*7 day*	*21 day*						
GNA	CELL	2(G)	2(G)	2(G)	3(G)	3	2(G)	0	1	1(G)	0
	CELL SURFACE	2	2	2	3	3	0	0	0	0	0
	PERI-LACUNAR	0	0	0	0	0	0	0	0	0	0
	MATRIX	0	0	0	0	0	0	0	0	0	0
PSA	CELL	2	3(G)	2	2	1	0	0	0	0	0
	CELL SURFACE	3	3	3	3	2	2	2	3	2	0
	PERI-LACUNAR	2	2	2	2	0	2	3	3	3	1
	MATRIX	3	2	2	3	0	0	2	2	0	0
LCA	CELL	2	2	2	2	2	0	0	0	0	0
	CELL SURFACE	3	3	4	3	2	0	2	2	0	0
	PERI-LACUNAR	2	3	2	3	2	0	3	3	2	2
	MATRIX	3	1	3	3	0	2	0	0	0	0
ePHA	CELL	2	2(G)	2	3(G)	3	3(G)	3	3	2	0
	CELL SURFACE	3	2	3	2	3	3	4	3	2	0
	PERI-LACUNAR	3	2	2	2	2	3	3	3	1	2
	MATRIX	2	2	3	3	0	2	2	2	0	0
lPHA	CELL	0	1	0	0	2	0	0	0	0	3(G)
	CELL SURFACE	2	1	2	2	2	0	0	0	0	0
	PERI-LACUNAR	0	0	0	0	0	0	0	0	0	0
	MATRIX	1	1	0	2	0	0	0	0	0	0
STA	CELL	2	2	2	2	3	2	2	2	3	3(G)
	CELL SURFACE	3	3	4	3	4	2	3	3	2	0
	PERI-LACUNAR	2	3	2	2	3	3	3	3	3	3
	MATRIX	3	2	3	2	2	2	2	2	2	0
LEA	CELL	2	3(G)	0	2(G)	2	0	0	2	2	0
	CELL SURFACE	3	3	3	3	2	0	2	2	2	0
	PERI-LACUNAR	2	3	0	3	2	3	3	3	3	3
	MATRIX	3	3	3	3	2	2	2	2	2	3
DSA	CELL	0	3(G)	1(G)	3(G)	3	2	2	2	2	3(G)
	CELL SURFACE	3	3	3	2	3	2	3	2	2	0
	PERI-LACUNAR	2	0	0	2	2	1	2	2	1	3
	MATRIX	3	2	2	3	1	2	1	2	2	3

The pattern of lectin binding in the caudal and cephalic portions of the intact embryonic sternum was generally closely similar to that found in the corresponding cultured chondrocytes and their matrices (Figure 7). Minor differences were that: (1) BSA-1B4 stained the caudal half (regions D, E, and F) of embryonic sternum, but not the cultured cells; (2) DBA did not stain the embryonic sternum, but stained the surface of cultured chondrocytes weakly; (3) LTA bound to the cephalic end of embryonic sternum, but to cultured 'caudal' chondrocytes; and (4) lPHA bound to cells only at the extreme ends of the embryonic sternum, but stained cells and some of the matrix in the cultures.

The blocking sugars produced complete inhibition of staining with most of the lectins, DSA and SBA having greatly reduced levels of staining. Addition of methyl α-D-mannopyranoside to SBA did not influence the intensity of the staining produced. Neuraminidase pretreatment greatly reduced the staining of SNA and MAA. α-Mannosidase

Table 3. Staining of cultured chick sternal chondrocytes and chick embryonic sternum by lectins that bind to terminal glycans. Key to areas: CELL, intracellular staining; CELL SURFACE, staining of the surface of the cells; PERI-LACUNAR, staining of the matrix immediately outside the lacunae of the chondrocytes; MATRIX, staining to the matrix excluding the peri-lacunar region. G, granular, intracytoplasmic staining. 0 represents no staining, 1 weak, 2 moderate, 3 strong and 4 very strong, intense staining.

Lectin	*Area*	*Cultured sternal chondrocytes*				*Embryonic sternum (regions)*					
		'Cephalic'		*'Caudal'*		*A*	*B*	*C*	*D*	*E*	*F*
		7 day	*21 day*	*7 day*	*21 day*						
AHA	CELL	1	1	0	0	0	0	0	0	0	0
	CELL SURFACE	2	1	1	1	0	0	0	0	0	0
	PERI-LACUNAR	0	0	0	0	0	0	0	0	0	0
	MATRIX	0	0	0	0	0	0	0	0	0	0
ECA	CELL	0	0	0	2(G)	1	0	0	0	0	0
	CELL SURFACE	3	2	3	2	0	0	0	0	0	0
	PERI-LACUNAR	0	2	2	0	1	0	0	0	0	0
	MATRIX	2	1	2	1	1	2	2	2	3	0
CTA	CELL	0	0	2	2	0	0	0	0	0	0
	CELL SURFACE	2	2	2	2	0	0	0	0	0	0
	PERI-LACUNAR	0	2	0	0	0	0	0	0	0	0
	MATRIX	1	0	0	1	0	1	1	0	0	0
HPA	CELL	2(G)	1(G)	1(G)	4(G)	1	0	0	1	2	2
	CELL SURFACE	0	0	1	1	0	0	0	1	2	2
	PERI-LACUNAR	0	0	0	0	0	0	0	1	2	2
	MATRIX	0	0	0	1	0	0	0	1	2	2
WFA	CELL	0	3(G)	0	3(G)	3	2	2	2	2	2
	CELL SURFACE	2	2	2	2	3	0	3	3	2	2
	PERI-LACUNAR	2	2	1	3	3	3	3	3	4	4
	MATRIX	3	3	3	4	3	3	3	2	3	3
SBA	CELL	1	1	1	2(G)	1	0	1	0	0	0
	CELL SURFACE	0	0	0	0	0	0	0	0	0	0
	PERI-LACUNAR	0	0	0	2	0	0	0	0	0	0
	MATRIX	0	0	0	2	0	2	1	0	0	0
MPA	CELL	0	0	0	1	2	0	0	0	0	0
	CELL SURFACE	2	1	2	2	0	0	0	0	0	0
	PERI-LACUNAR	1	0	0	0	0	0	0	0	0	0
	MATRIX	1	0	1	0	0	0	2	2	2	0
DBA	CELL	0	0	0	0	1	0	0	0	0	0
	CELL SURFACE	1	1	1	1	0	0	0	0	0	0
	PERI-LACUNAR	0	0	0	0	0	0	0	0	0	0
	MATRIX	0	0	0	1	0	0	0	0	0	0
UEA-1	CELL	0	0	0	0	1	0	0	0	0	0
	CELL SURFACE	1	2	1	2	0	0	0	0	0	0
	PERI-LACUNAR	0	2	0	0	0	0	0	0	0	0
	MATRIX	1	0	0	2	0	0	0	0	0	0
LTA	CELL	0	1	0	0	2	2	1	0	0	0
	CELL SURFACE	1	1	1	2	0	0	0	0	0	0
	PERI-LACUNAR	0	1	0	2	0	2	1	0	0	0
	MATRIX	0	0	0	3	0	0	0	0	0	0
BSA-1B4	CELL	0	0	0	0	1	0	0	2	2	2
	CELL SURFACE	0	0	0	0	0	0	0	0	0	0
	PERI-LACUNAR	0	0	0	0	0	0	0	0	0	0
	MATRIX	0	0	0	1	0	0	0	2	2	2

Table 3. (Continued)

Lectin	*Area*	*Cultured sternal chondrocytes*				*Embryonic sternum (regions)*					
		'Cephalic'		*'Caudal'*		*A*	*B*	*C*	*D*	*E*	*F*
		7 day	*21 day*	*7 day*	*21 day*						
MAA	CELL	0	0	0	0	2	1	0	1	2	0
	CELL SURFACE	2	2	2	2	0	0	0	0	0	0
	PERI-LACUNAR	0	2	2	2	0	0	0	0	0	0
	MATRIX	2	0	2	2	0	1	1	1	2	0
SNA	CELL	0	0	0	2(G)	0	0	0	2	0	0
	CELL SURFACE	0	2	2	2	0	0	0	0	0	0
	PERI-LACUNAR	0	0	0	0	0	0	0	0	0	0
	MATRIX	1	2	2	3	0	0	1	2	1	1

pretreatment also greatly reduced the staining with GNA and partially reduced the staining with ePHA and lPHA. α-L-Fucosidase pretreatment stopped UEA-1 from staining.

Discussion

Specificity of the lectins was confirmed by the control studies using blocking sugars and enzyme pretreatments. EPHA and lPHA bind to specific mannose-containing oligosaccharides in which mannose is not terminal [16, 17] and, therefore, they are not degraded by the α-mannosidase used in the control experiments (which is an exoenzyme). This explains the residual staining found with ePHA and lPHA after α-mannosidase treatment. The lack of any inhibition of staining with SBA, on incubation with methyl α-D-mannopyranoside, shows that endogenous lectins are not attaching to the mannose of the glycan of SBA [18]. Some of the lectins used (BSA-$1B_4$, ECA, CTA, DBA and WFA) are of a set of lectins that contain similar N-linked oligosaccharides with α-mannosyl termini [19], yet these gave totally different staining patterns from each other (*eg* compare that produced by BSA-$1B_4$ with that of DBA). This confirms that endogenous lectins are not contributing appreciably to the staining observed in this study.

The chondrocyte cultures, including both the cultured cells and their matrices, differed in their lectin binding between the two cell subpopulations, so demonstrating differences either in their content of glycan or in its arrangement. Complex variations were also seen, over time, in the binding of lectins to cell membranes, cytoplasmic granules and to the various regions of the matrices. Embryonic sternum demonstrated an unexpected complexity in the distribution of accessible glycans both within the overall structure of the sternum and, locally, in relation to the individual cells and matrices. The more mature sternum also revealed regional variations in lectin binding, with differences occurring in the staining of territorial and interterritorial matrix between adjacent areas of cartilage.

Of the lectins used in this study, the majority bind either exclusively, or very predominantly, to saccharide sequences which include the non-reducing terminal sugars of glycans and they interact minimally or not at all with wholly 'internal' sequences. Hence, most of the lectins used will have stained in proportion to the numbers of available non-reducing termini, irrespective of the length of the glycan chains that bore them. The exceptions are PSA, LCA, ePHA and lPHA, which bind to parts of the core sequences and outer chains of various subsets of complex N-glycans, and STA, LEA and DSA, which have varying affinities for di-*N*-acetyl chitobiose and *N*-acetyllactosamine, so that STA, at least, may bind to some forms of keratan sulphate.

Those lectins which bind to subsets of N-glycans (GNA, PSA, LCA, ePHA and lPHA) [20, 21] stained the cultured chondrocytes more strongly than the sternum. Previous studies, using Concanavalin A, which interacts with a wide range of N-glycans and with glycogen [22, 23], found that it bound to chondrocytes, especially of the superficial zone of articular cartilage, and to the cytoplasmic vesicles of cultured chondrocytes [10, 24–26]. In this study GNA, which is more selective and binds to Manα1,3Manα1- termini and not to α-glucosyl termini [27], stained glycans of intracellular granules, which were probably secretory vesicles or lysosomes. These were not present in the spindle-shaped cells early in culture, only appearing as they started to produce an extracellular matrix. GNA only bound intracellularly, indicating that high mannose N-glycans were restricted to the interior of the cells or, if external, were cryptic.

The interpretation of the binding of PSA, LCA, ePHA and lPHA to various subsets of complex N-glycans has been discussed elsewhere [17, 28, 29].The stronger staining with PSA and LCA than with lPHA suggests that the majority of non-bisected N-glycans were bi-antennary and that tetra-antennary forms were rare. Strong staining with ePHA is probably a reflection of a high content of bisected bi/tri-antennary N-glycans, especially in matrix. The higher level

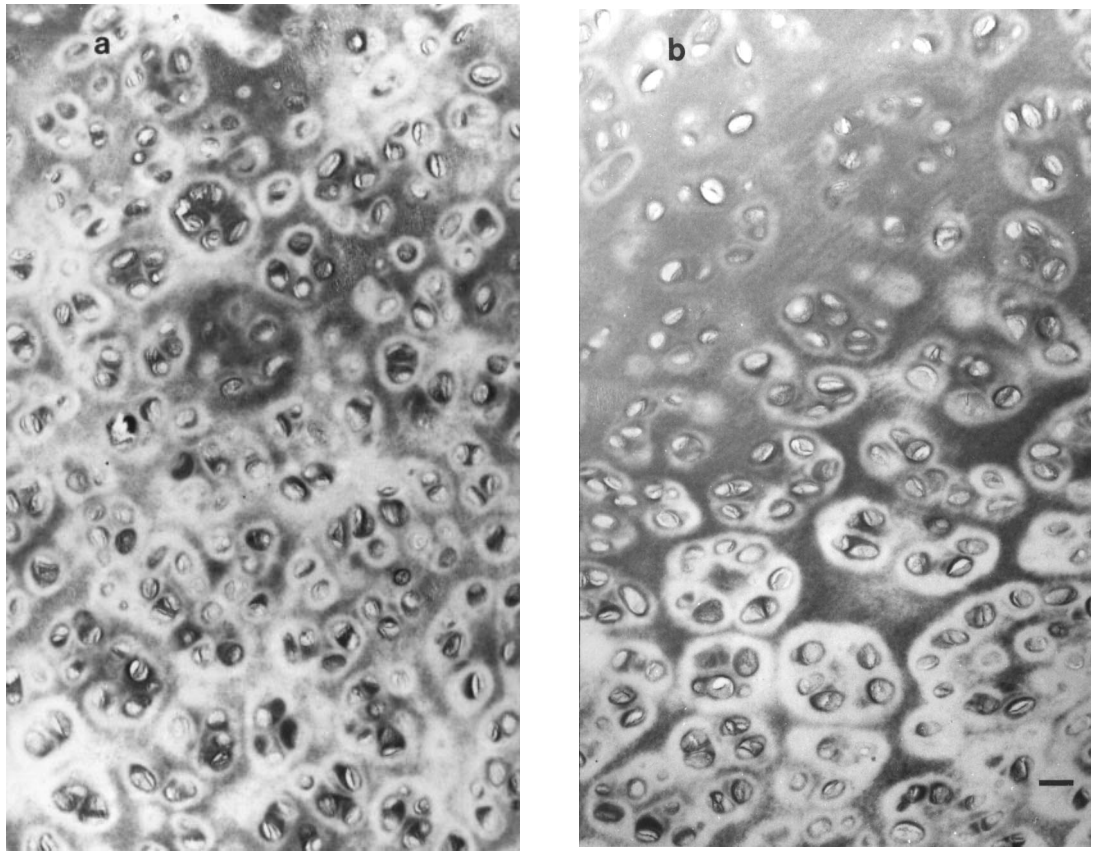

Figure 4. Six-week-old chick sternum stained with (a) WFA and (b) STA illustrating regional variation in areas of matrix stained. Bar = 20 μm.

of intracellular stain given by PSA and LCA in the cultured cells, as opposed to the sternum, may reflect both the numbers of intracellular granules containing high mannose glycans in the former, as well as differences in the dynamics of translocation of N-glycans through endomembranes, their processing and outer chain synthesis.

ECA, CTA and DSA are all probes of terminal *N*-acetyllactosamine [30, 31]. ECA and CTA bound more strongly both to cells and matrix of cultured chondrocytes than to the embryonic sternum, where staining was almost exclusively to the matrix. ECA stained more strongly than CTA in all the tissues tested and especially in the mature sternum. ECA and DSA showed opposing trends in their staining of the perilacunar matrix of cultured chondrocytes, indicating a probable shift from one subset of glycans carrying terminal *N*-acetyllactosamine to another. ECA and CTA differ from each other in their interactions with *N*-acetyllactosamine in repeated sequences and multiple termini [32]. They have binding requirements which overlap with those of RCA_{120} [33], in that RCA_{120} can tolerate a 6-sialyl substituent on the β-galactosyl residue, but at no other position [34], while ECA will tolerate an α-galactosyl substituent only at C3 of the β-galactose [32, 35]. RCA_{120} has been reported to stain the perinuclear region of cultured chondrocytes from chick embryo sternum [24]. In our study ECA and CTA bound to cultured 'caudal' chondrocytes, but cultured 'cephalic' chondrocytes did not stain.

There were marked discrepancies in staining pattern between STA and DSA, with LEA resembling DSA. Hence STA and DSA were probably detecting largely unrelated glycans, with the latter binding predominantly to subsets of *N*-acetyllactosamine termini.

HPA, WFA, SBA and DBA all require terminal residues of *N*-acetyl galactosamine, but differ in their other, specific, structural requirements [36–38]. These lectins gave varied binding patterns, some binding to one subpopulation or stage of cells in culture, others to selected areas of the embryonic sternum and they varied widely in the intensity of their staining, indicating the presence of several subsets of sequences with terminal α-*N*-acetyl galactosamine which were expressed very variably.

MPA has a high affinity for Galβ1,3GalNAcα1- terminal disaccharides, but not for most other β-galactosides; it can also bind to α-*N*-acetyl galactosamine [39, 40]. It stained only the surfaces of the cultured chondrocytes and the matrix of the mid-sternal region. AHA similarly binds preferentially to Galβ1,3GalNAcα1-, but it can also attach to Galβ1,4GlcNAcβ1- and Galβ1,3GlcNAcβ1- termini

[41, 42]. In previous studies, AHA was applied to chick sternal chondrocytes cultured on gelatin-coated dishes and stained the Golgi apparatus [43]. In our hands it bound weakly to the cells, but not to the matrix of the cultures, and was totally negative in the sterna. The weak cellular staining may have been attributable to *N*-acetyllactosamine and is consistent with the binding of ECA. The true distribution of Galβ1,3GalNAcα1- was, therefore, very restricted.

UEA-1 binds to Fucα1,2Galβ1,4GlcNAcβ1- in 'conventional' glycoprotein glycans and in related forms of keratan

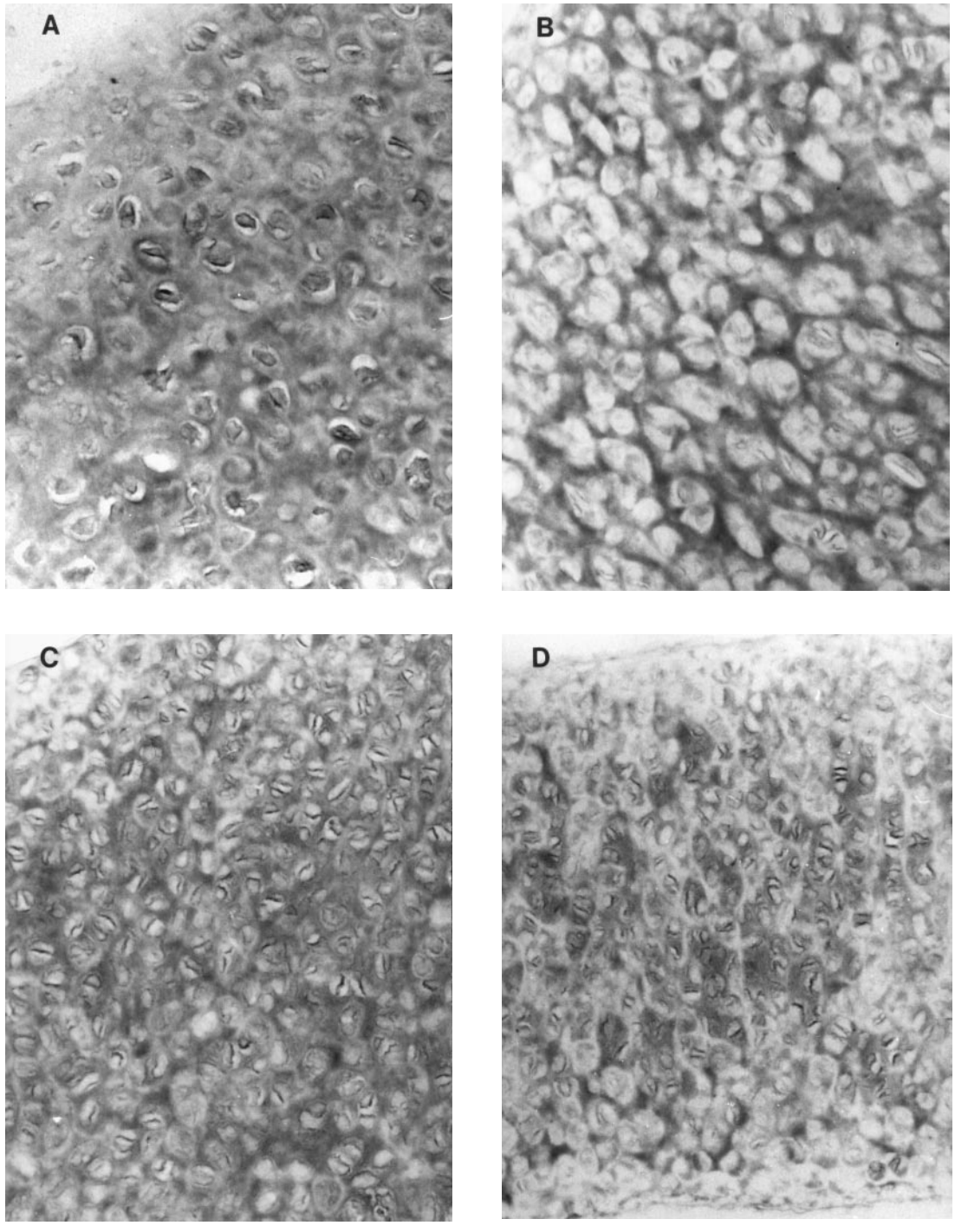

Figure 5. Embryonic chick sternum stained with the lectin WFA illustrating the variation found in the staining pattern to the different regions of the sternum, A to F. Bar = 20 μm.

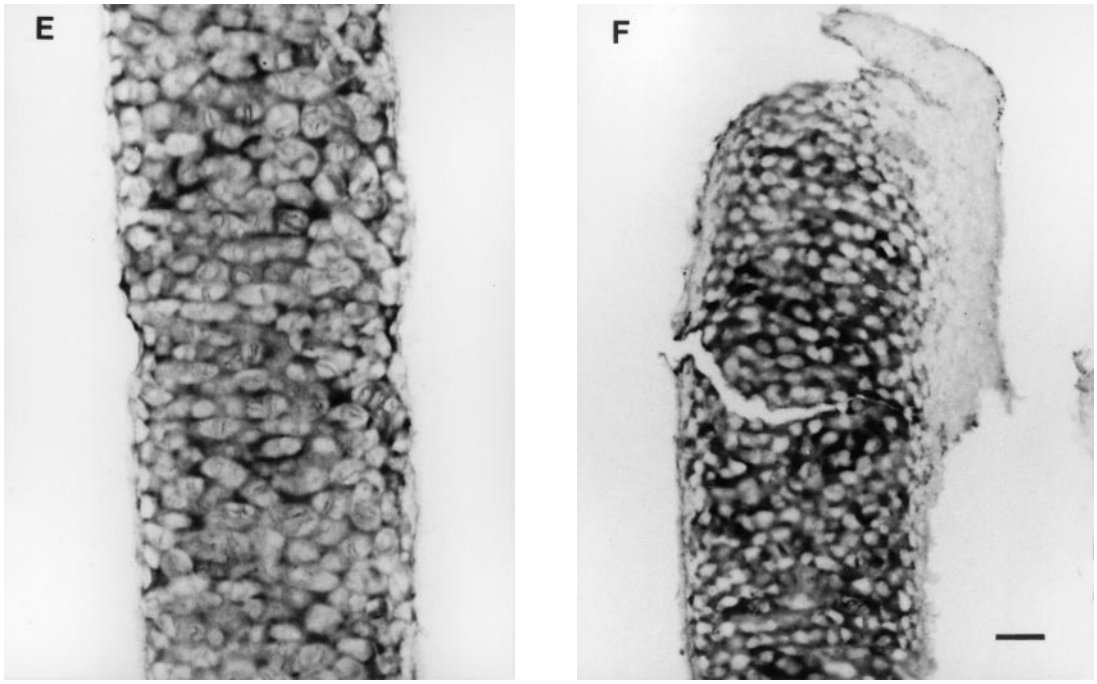

Figure 5. (Continued).

sulphate, while LTA can interact with clusters of fucosyl residues, or the Fucα1,6(-4GlcNAcβ1,4)GlcNAcβ1- sequence in core-fucosylated N-glycans [29]. Both gave much stronger staining at day 21 than in earlier cultures, suggesting a late upregulation of more than one type of fucosyl transfer in the cultures.

Staining with BSA-1B4 was limited to the caudal end of the embryonic sternum, indicating that Galα1,3Galβ1, 4GlcNAc- was very sparingly and locally expressed.

Sialyl termini were detected, both in α2,6- and α2,3-linkage with SNA and MAA respectively, in the caudal portion of the immature sternum and in the 'caudal' chondrocytes in culture. Both lectins stained the 'caudal' cells more strongly than their 'cephalic' counterparts, implying possible differences in at least two sialyl transfers. Both types of sialyl linkage are known at the non-reducing terminal in 'cap' sequences of mammalian keratan sulphate [44]. Chicken aggrecan, like its mammalian counterpart, contains a keratan sulphate domain, though in the bird it lacks the repeat sequences seen in bovine and human aggrecan [45]. Hence it is possible that the changes detected with SNA and MAA are reflecting the abundance of the capped structure of keratan sulphate, rather than a simple regulation of sialyl transfer.

These observations show that the matrix of chick sternal cartilage is highly inhomogeneous and shows numerous subtle differences in glycan expression both between the various regions of immature and mature sterna and between their territorial and interterritorial matrices. The chondrocytes from the caudal and cephalic ends of the chick embryo sternum had previously been shown to have fundamental, biochemical differences and our study extends them to several new classes of glycan. The differences in glycan expression involve several zones of the matrices, the cell surface and intracellular organelles, so that each form of chondrocyte has a distinct cellular and matrix 'glycotype' that is largely preserved in culture. This implies that the chondrocytes are already committed to particular differentiation pathways before they are isolated, rapidly revert to type if their matrix is removed and are otherwise rather insensitive to alterations in levels of growth factors in their environment.

The pattern of lectin binding to the intact embryonic sternum demonstrated an unexpected complexity, with at least six distinct regions being revealed. This complexity was also reflected in the mature sternum. In studies of the development of hyaline cartilage of the rat, Zschäbitz *et al.* [46] also found that the patterns of lectin binding to the developing cartilage varied both with time and position within the tissue. The detailed pattern of staining diverged substantially from that seen here, however, with nine of the 14 lectins common to both studies showing differences. In general the range of glycans expressed in the chick appeared greater than in the rat.

The weak background staining seen in the cultures with several of the lectins was largely attributable to glycoproteins derived from the foetal calf serum present in the culture medium. The 'halo-like' loss of this background stain

around the cultured chondrocytes could have been the result either of proteolysis or of glycosidase activity, and is an indicator of the ability of chondrocytes to degrade or modify surrounding glycoprotein up to a considerable distance from the cells, as well as to deposit new matrix.

Many components of the matrix contain oligosaccharide sequences to which various lectins can bind, though the abundance of these need not be related to the total amount of glycan present and similar glycans may occur on different peptide cores. The striking degree of spatial restriction in the staining patterns of some lectins, both in the sterna and,

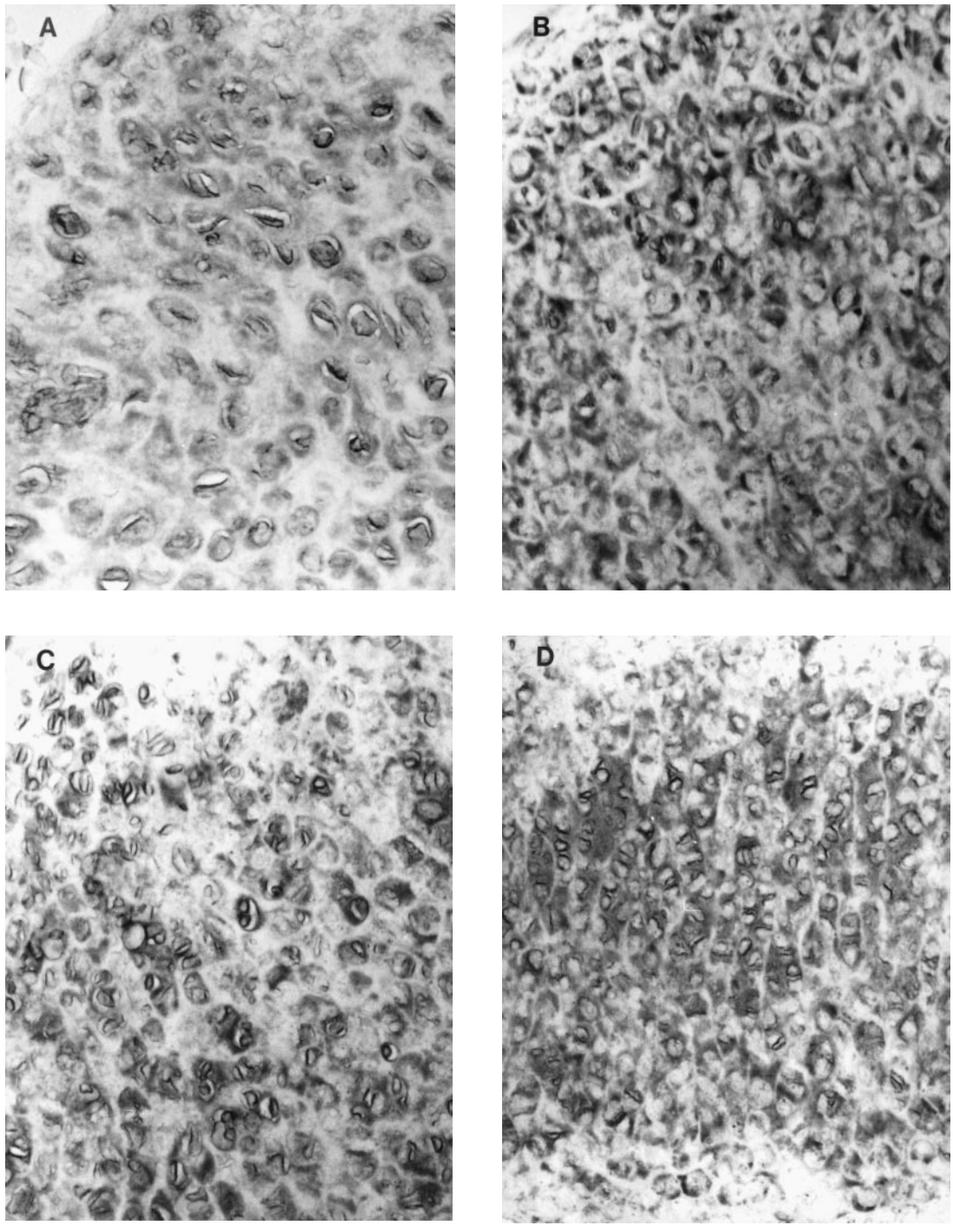

Figure 6. Embryonic chick sternum stained with the lectin STA illustrating the variation found in the staining pattern to the different regions of the sternum, A to F. Bar = 20 μm.

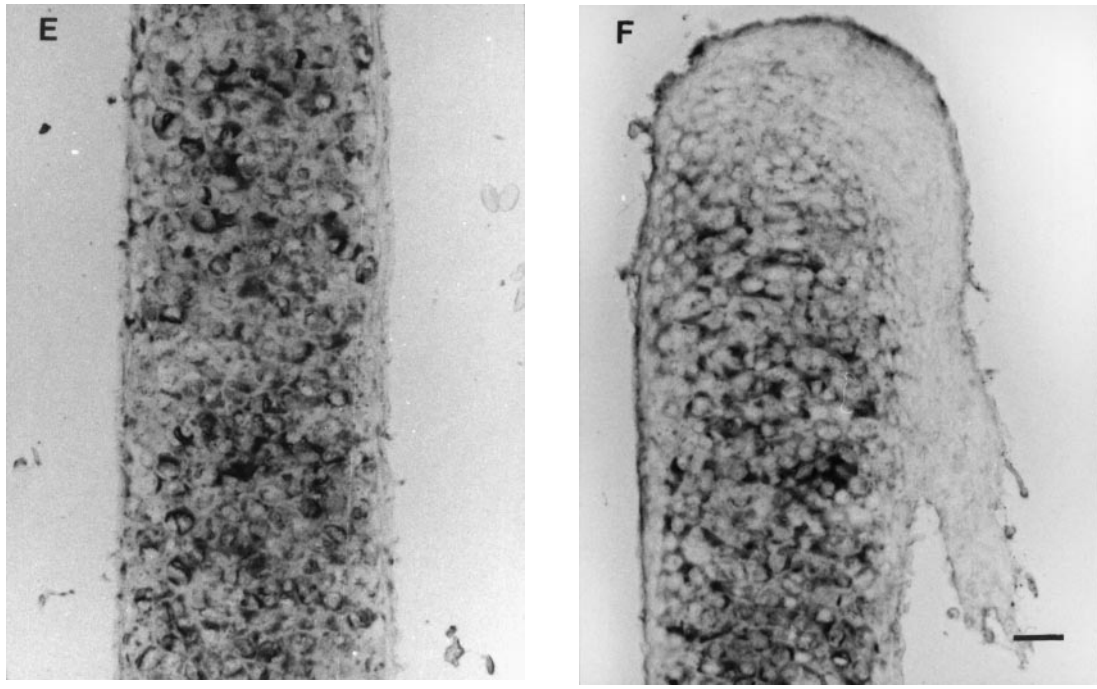

Figure 6. (Continued).

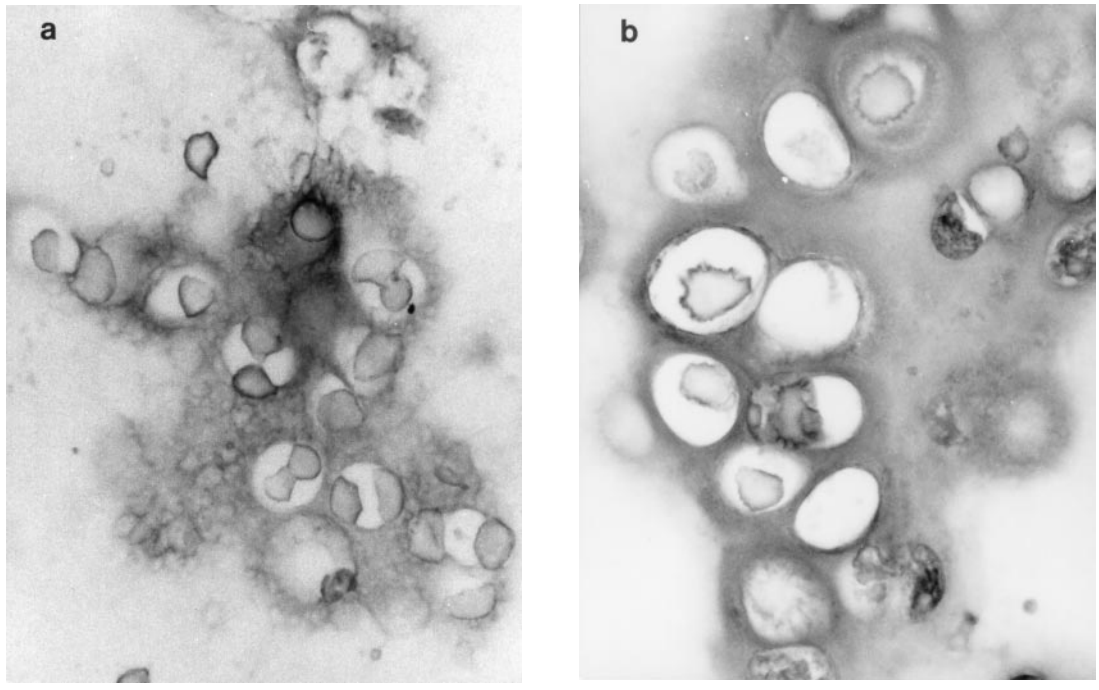

Figure 7. Cultured chick sternal chondrocytes stained with WFA from the caudal end of the sternum (a) and (b), and from the cephalic end of the sternum (c) and (d). (a) and (c) were grown for 7 days and (b) and (d) were grown for 21 days. Bar = 10 μm (see overleaf).

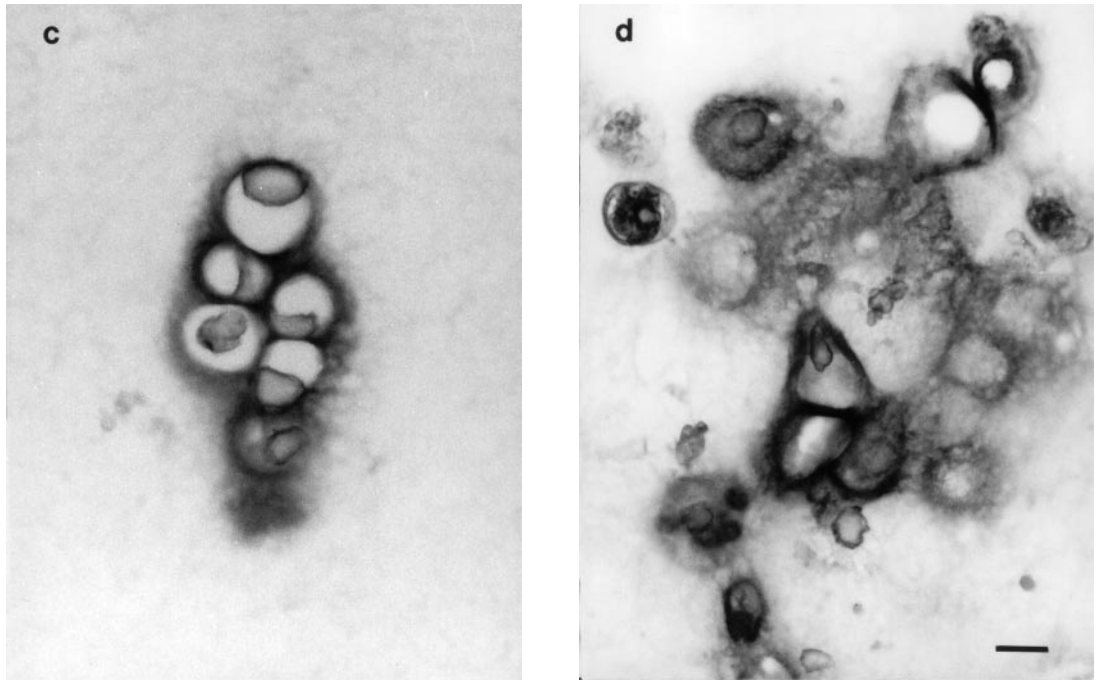

Figure 7. (Continued).

at specific times, in the cultures, implies that particular glycans come to occupy particular locations in the developing matrix. Whether these glycans are influencing the pattern of matrix assembly, or whether they are merely reflecting it, is unknown. Many proteins of both matrix and cell surface membranes however, contain peptide sequences which are characteristic of the C-type animal lectins and in some cases these appear to be functional, while others might only become activated by partial degradation of the matrix. Hence partial fragmentation of the matrix could release both glycans, as glycopeptides, and molecules for which they are ligands. There is, therefore, the intriguing possibility that many of the smaller non-repetitive glycans of the matrix are informational, either in the sense that they help to specify patterns of assembly or are vehicles whereby the degradation of matrix feeds back upon and regulates the cells that produce it. Such a proposition is consistent with the concept that glycans have a wide and general role in information transfer within biological systems [47] and with the known effects of intact proteoglycan and glycosaminoglycan upon the phenotype of cells of mesenchymal origin [48].

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