Acid mucopolysaccharides in the development of the Pacific great skate, *Raja binoculata*

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INTRODUCTION

Existing histochemical techniques permit the identification of AMPS (Dorfman, 1963; Saunders, 1964; Scott, Quintarelli & Dellova, 1964; Spicer, 1960, 1963; Szirmai, 1963; Yamada, 1963a, b, 1964; Zugibe, 1962, 1963) and considerable information is available on the AMPS constitution of specific tissues in adult organisms (Anno, Seno & Kawaguchi, 1963; Mathews & Hinds, 1962; Szabo & Roboz-Einstein, 1962). However, it has not yet been shown that it is possible to characterize AMPS specifically by histochemical methods in embryos or that the chain of events leading to specific AMPS localization in adult organisms can be ascertained histochemically during embryonic development. In this paper the following abbreviations are used: AMPS, acid mucopolysaccharide; CPB, cetyl-pyridinium bromide; PAS, periodic acid-Schiff reaction; C-S-A/C, chondroitin sulphate A and/or chondroitin sulphate C; C-S-B, chondroitin sulphate B; ABPAS, combined Alcian blue-PAS stain; ECM, extracellular material.

Previous observations in this laboratory of embryo *Raja binoculata* yolk stalks indicated that sufficient AMPS are present to permit such a study (McConnachie & Ford, 1964). This report provides information, obtained by histochemical means, concerning the AMPS occurring throughout the embryonic development of the Pacific (great) skate, *Raja binoculata.*

MATERIALS AND METHODS

Embryonic materials

*Raja binoculata* embryos were obtained in egg cases by trawl at 16 fm off Tsawassen, in the Straits of Georgia, B.C., and were maintained in the laboratory in 30 gal tanks of circulating sea water at 12 °C. The incubation times of various rays ranges from 9 to 15 months (TeWinkel, 1960, 1963; Libby & Gilbert, 1960;

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but is not known for *R. binoculata*. However, the rate of development of this species is slow so that a succession of developmental stages was obtained which ranged from cleaving eggs through hatching embryos.

Embryos were isolated, fixed in CPB-formalin for 48 h at room temperature (Williams & Jackson, 1956), dehydrated through ethanol, embedded in paraffin, sectioned at 8 µ and mounted on glass slides.

**Staining**

Sections of embryos were treated with two or three of the following mucopolysaccharide stains: (a) Azure A, 0.1 % at pH 1.5 and pH 4.0 to demonstrate respectively strongly acidic sulphated AMPS (chondroitin sulphates) and weakly acidic non-sulphated AMPS (hyaluronic acid) according to the method of Szirmai (1963); (b) alcian blue-PAS to demonstrate AMPS and neutral polysaccharides as used by Spicer (1960); (c) acridine orange to demonstrate respectively hyaluronic acid, the chondroitin sulphates and heparin according to the method of Saunders (1964).

**Enzyme treatments and chemical blocking and unblocking**

Sections of *Raja* embryos were treated for 3 h at 37°C with testicular hyaluronidase (Nutritional Biochemicals Corp.) 1 mg/ml in 0.85 % saline (Pearse, 1961) prior to staining to assist in localizing hyaluronic acid, C-S-A and/or C-S-C which are hydrolysed by the action of this enzyme (Walker, 1961).

When desired, hyaluronic acid was extracted from sections by a 10 min treatment with 0.3 M-NaCl in 0.01 M acetic acid at room temperature while treatment of similar sections with 0.6 M-NaCl in 0.01 M acetic acid extracted both hyaluronic acid and the chondroitin sulphates (Kelly, Bloom & Scott, 1963; Saunders, 1964).

To remove glycogen, *Raja* sections were incubated in 0.1 % commercial malt diastase in glass distilled water at 37°C for 30 min (Yamada, 1963a). Oligosaccharides and labile polysaccharides were partially removed by prolonging the incubation from 3 to 24 h (Pearse, 1961).

A methylation-demethylation sequence treatment (Fisher & Lillie, 1954; Yamada, 1964) was used to block carboxyl and sulphate groups by methylation and to selectively restore carboxyl groups by demethylation prior to staining with ABPAS and Azure A. The methylation–demethylation sequence was useful in conjunction with Azure A staining at controlled pH values in assessing the relative staining due to carboxyl and sulphate groups respectively. ABPAS results were not consistent when used with this treatment.

An acetylation–deacetylation treatment (Pearse, 1961) was used to first block all PAS reactivity and then to restore selectively PAS staining to hydroxyl groups of embryo sections prior to ABPAS staining. This treatment was useful only in locating glycogen and neutral polysaccharides in conjunction with PAS staining and diastase digestion.
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Specificity of staining methods

Satisfactory agreement between Azure A, ABPAS and Acridine orange staining was obtained in cleaving stages through neurulae. Alcian blue responses showed variation with the first appearance of sulphated AMPS in post neurulae (17-18 mm embryos). Methylation failed to block alcianophilia and subsequent demethylation often abolished alcianophilia entirely. A similar loss of alcianophilia and azurophilia was frequently produced by deacetylation following acetylation. Yamada (1964) observed such reversals of expected staining of known model sulphated polysaccharides using Alcian blue and PAS staining. He suggested that Alcian blue staining was not necessarily dependent on acidic groups and that strongly acidic groups may be affected by the alkali used in demethylation. A revised method for Alcian blue (Scott et al. 1964) apparently has overcome such difficulties, allowing more specific blocking and unblocking. Azure A and Acridine orange results were in agreement throughout all stages of embryonic development.

Characterization of AMPS was based on lability to hyaluronidase, relative acidity, resistance to salt extraction and specific blocking and unblocking of chemical groups. With the techniques employed, identification of glycogen, neutral polysaccharides, hyaluronic acid, C-S-A/C and C-S-B was achieved. Identification was least difficult in the earliest developmental stages.

RESULTS

Development of extracellular material

Cleavage stages

Cleaving eggs showed very little azurophilia but, in contrast, considerable PAS reactivity appeared which was labile to diastase digestion only after 4-6 h. A gradient was observed in PAS reactivity, which was most intense in the deeper portions of cells and adjacent to cell membranes and least intense in the superficial portions of cells (Plate, fig. A). This gradient could be indicative of fixation polymerization of glycogen (Pearse, 1961), but this is doubtful because of its resistance to prolonged diastase digestion. Azurophilia showed a similar gradient and was completely labile to hyaluronidase digestion. Acetylation abolished all staining and subsequent deacetylation restored PAS reactivity.

These results indicated the predominance of neutral and slightly acidic polysaccharides in cleavage stages with the possible initial presence of slight amounts of hyaluronic acid.

Early neurulae (2-4 mm embryo)

Extracellular staining associated with mesenchyme cell processes appeared primarily as a layer on the ventral surface of the neural ectoderm, and the epidermis, and was designated as extracellular material (ECM). Thin strands of
ECM extended from the ectoderm and epidermis to the underlying mesoderm, from the mesoderm to the underlying endoderm (Plate, fig. B), and a thin sheet surrounded the notochord. Azure A staining showed that dye binding was through carboxyl and possibly hydroxyl groups and the complete lability to hyaluronidase (Table 1) indicated that this ECM was partially hyaluronic acid. Concurrent Alcian blue and PAS reactions indicated the participation of neutral polysaccharides in ECM staining. An acetylation–deacetylation sequence followed by ABPAS staining demonstrated the presence of intracellular neutral and slightly acidic polysaccharides in higher concentration than the AMPS of ECM.

Table 1. *Staining of extracellular material (ECM)*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ECM of 2–4 mm embryo</th>
<th>ECM of 6–7 mm embryo</th>
<th>Basement membrane ECM of 17–18 mm embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azure A, pH 4·0</td>
<td>0+ to +++.</td>
<td>M + +</td>
<td>M + + +</td>
</tr>
<tr>
<td>Azure A, pH 1·5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hyaluronidase, Azure A, pH 4·0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Methylation, Azure A, pH 4·0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Methylation-demethylation, Azure A, pH 4·0</td>
<td>0± to 0+ +</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>ABPAS</td>
<td>ABP + + + + +</td>
<td>AB + + + +</td>
<td>AB + + + +</td>
</tr>
<tr>
<td>Acetylate</td>
<td>–</td>
<td>N.A.</td>
<td>AB + + + +</td>
</tr>
<tr>
<td>ABPAS</td>
<td>–</td>
<td>N.A.</td>
<td>AB + + + +</td>
</tr>
<tr>
<td>Acetylate–deacetylate ABPAS</td>
<td>–</td>
<td>N.A.</td>
<td>AB + + + +</td>
</tr>
<tr>
<td>Saunders 1</td>
<td>N.A.</td>
<td>+ + + +</td>
<td>N.A.</td>
</tr>
<tr>
<td>Saunders 2</td>
<td>N.A.</td>
<td>–</td>
<td>N.A.</td>
</tr>
<tr>
<td>Saunders 3</td>
<td>N.A.</td>
<td>–</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

*Neurulae (6–7 mm embryo)*

The ECM was now much more extensive than in the above earlier stages. It occurred in association with mesoderm cell processes between mesoderm and any other adjacent tissue but not between somatic and splanchnic mesoderm (Plate, fig. C). Staining results indicated that at this developmental stage ECM was entirely AMPS (Table 1), in a state of greater polymerization, as shown by the appearance of metachromacy in Azure A staining. The presence of hyal-
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Acidic mucopolysaccharides of the skate were indicated by the intense Acridine orange staining and by the lability of Azure A staining to hyaluronidase.

Cytoplasmic azurophilia in the 6–7 mm embryo was not intense and was labile to hyaluronidase digestion. In mesoderm and neural ectoderm cells the hyaluronic acid content was greater than the neutral polysaccharide content as indicated by increased azurophilia and alcianophilia and decreased PAS reactivity.

Post-neurulae (17–18 mm embryo)

In these developmental stages ECM was not present to the extent observed in the previous stages but was restricted to basement-membrane-like layers between mesoderm and endoderm of organ structures, e.g. heart and gut tube. In addition a layer involving mesoderm alone surrounded the developing mesonephric tubule (Plate, figs. D, E). Staining results (Table 1) still indicated hyaluronic acid to be the primary component of these structures.

In comparison to previous stages, there was increased cytoplasmic azurophilia and alcianophilia in trunk mesoderm which suggested some AMPS content.

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**EXPLANATION OF PLATE**

The linear scale represents 0.5 mm in fig. A and 0.1 mm in figs. B to H.

Fig. A. Section through an early blastodisc showing the gradient of PAS reactivity which persists following 2 h digestion in diastase. (ABPAS.)

Fig. B. Cross-section of 2–4 mm early neurula showing the ECM (e) layer on the ventral surface of the neural ectoderm and the strands of ECM extending from the mesoderm to the underlying endoderm. (Azure A, pH 4.0.)

Fig. C. Cross-section through midgut region of 6–7 mm neurula showing the extent of the ECM layers between adjacent tissues, i.e. between ectoderm and mesoderm, between mesoderm and endoderm, around the notochord, but not between somatic and splanchnic mesoderm. (Azure A, pH 4.0.)

Fig. D. Cross-section of 17–18 mm embryo in mid-trunk region showing hyaluronic acid in basement-membrane-like structure around the developing mesonephric tubules (m). Several layers of AMPS matrix are evident in portions of the notochord sheath (s). (Azure A, pH 4.0.)

Fig. E. Section through gut region of 17–18 mm embryo showing hyaluronic acid in basement-membrane-like layer between endoderm and mesoderm of developing gut (g). (Azure A, pH 4.0.)

Fig. F. Section through epidermis of 140 mm embryo showing C-S-B in unicellular mucous glands (u). (Hyaluronidase, Azure A, pH 1.5.)

Fig. G. Section through mid-trunk region of 40 mm embryo showing the layered structure of the notochord sheath. The AMPS matrix forming the outer layer of the sheath is notably more extensive laterally than it is dorsally adjacent to the neural tube (t), or ventrally. The inner layer (i) is faintly orthochromatic and the middle layer shows an extremely dense metachromasy. (Azure A, pH 4.0.)

Fig. H. Section through the branchial region of a 70 mm embryo showing precartilage matrix (c) of the parachordal elements extending dorsolaterally from the notochord sheath. (0.3 M-NaCl, Azure A, pH 1.5.)
associated with the derivation of basement membrane layers. It is notable that in both trunk mesoderm and endoderm of this stage, the acetylation–deacetylation sequence induced an alcianophilia which was not present in control sections. This is in agreement with Yamada’s (1963b) suggestion that Alcian blue staining may involve groups other than carboxyl and sulphate.

In later developmental stages AMPS were generally restricted to association with the notochord sheath, the associated sheath mesenchyme (fibroblasts) and the trunk mesoderm. ECM or basement-membrane-like layers did not appear in developmental stages later than 17–18 mm.

One exception to this distribution was found in the 40 mm embryo epidermis where faint azurophilia at pH 1·5 persisted following hyaluronidase digestion, indicating some C-S-B. However, this observation was not supported by positive Acridine orange staining and is not definite at this developmental stage. Both trunk and heart mesoderm may contain hyaluronic acid and C-S-A/C as minor components, as slight azurophilia is observed at pH 1·5, but this also is not supported by Acridine orange staining.

In the 70 mm embryo AMPS was indicated by azurophilia at pH 1·5 in epimyocardial cells, mesonephros cells and, particularly, in some neuron-like cells in the ventro-lateral mantle area of the neural tube. An intense azurophilia at pH 4·0, a weaker azurophilia at pH 1·5 and a complete lability to hyaluronidase indicated hyaluronic acid and lesser quantities of C-S-A/C which have been reported to be present in neurons of bovine brain and spinal cord (Szabo & Roboz-Einstein, 1962).

In the 140 mm embryo (immediate prehatching stage) unicellular epidermal mucous glands showed intense metachromasy in their lumina at pH 1·5 following hyaluronidase digestion (Plate, fig. F). This material appeared to be C-S-B as it was extracted by 0·6 M-NaCl, was not labile to hyaluronidase digestion, and did not stain following methylation and demethylation.

**Notochord sheath development**

In the 2–4 mm embryo the notochord sheath was less than \( \frac{1}{2} \mu \) in thickness and was identical with the ECM in other embryonic areas, being composed of neutral polysaccharides and hyaluronic acid. In the 6–7 mm embryo the notochord sheath was 1–1·2 \( \mu \) thick, and contained less polysaccharide and more hyaluronic acid as shown by the appearance of metachromasy and the resistance of alcianophilia to diastase (Table 2). Also, a slight azurophilia at pH 1·5 and positive Acridine orange staining indicated the presence of some C-S-A/C. In the 17–18 mm embryo there were two or three thin layers of precartilage matrix around the sheath (Plate, fig. D) which were metachromatic with Azure A at pH 4·0, while at pH 1·5 there were only two demonstrable layers, the inner orthochromatic and the outer metachromatic. Hyaluronidase removed all azurophilia; hence the two layers which stained at pH 1·5 contained C-S-A/C in significant concentration in comparison to earlier stages. Mesenchyme cells
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adjacent to the notochord sheath contained hyaluronic acid, as they showed metachromasy at pH 4-0, lability to hyaluronidase and an absence of staining at pH 1-5.

The structure of the 40 mm embryo notochord sheath was much more complex, since three different layers could be demonstrated (Plate, fig. G). The inner layer formed the outer surface of the notochord and was PAS-positive, orthochromatic with Azure A at pH 4-0, not labile to hyaluronidase and labile to diastase; all of which indicate a slightly acidic polysaccharide composition. The middle layer was 4–5 μ thick, and contained hyaluronic acid, C-S-A/C and C-S-B as shown by persistent metachromasy following hyaluronidase digestion. Results obtained with Acridine orange indicated that most of the staining was due to the chondroitin sulphates. The third layer consisted of an AMPS matrix between the sheath and the adjacent fibroblasts which was more extensive laterad than dorsad or ventrad. C-S-A/C was the major component and hyaluronic acid the minor component of this matrix.

In the 70 mm embryo, cartilage development had begun with the growth of neural and hemal arches from the corners of the sheath and the establishment of branchial and chondrocranial skeletal elements. These all appeared as a concentration of C-S-A/C and hyaluronic acid in a uniform matrix between aggre-

Table 2. Staining of notochord sheaths*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2–4 mm embryo</th>
<th>6–7 mm embryo</th>
<th>17–18 mm embryo</th>
<th>40 mm embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azure A, pH 4-0</td>
<td>0+ ++ +</td>
<td>M + + +</td>
<td>M + + +</td>
<td>0+ + + + to M + + +</td>
</tr>
<tr>
<td>Azure A, pH 1-5</td>
<td>–</td>
<td>0±</td>
<td>0± to M±</td>
<td>M + + to M ±</td>
</tr>
<tr>
<td>Hyaluronidase, Azure A, pH 4-0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>– to 0±</td>
</tr>
<tr>
<td>Hyaluronidase, Azure A, pH 1-5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>– to 0±</td>
</tr>
<tr>
<td>Methylation, Azure A, pH 4-0</td>
<td>–</td>
<td>N.A.</td>
<td>N.A.</td>
<td>–</td>
</tr>
<tr>
<td>Methylation–demethylation, Azure A, pH 4-0</td>
<td>0± to ++</td>
<td>N.A.</td>
<td>N.A.</td>
<td>– to 0+</td>
</tr>
<tr>
<td>Diastase, Azure A, pH 4-0</td>
<td>0+ to + + +</td>
<td>M +</td>
<td>N.A.</td>
<td>M ± to M + +</td>
</tr>
<tr>
<td>ABPAS</td>
<td>ABP+ + + +</td>
<td>ABP+ + + +</td>
<td>N.A.</td>
<td>ABP + + +</td>
</tr>
<tr>
<td>Diastase, ABPAS</td>
<td>AB + to</td>
<td>ABP + +</td>
<td>ABP+ + + +</td>
<td>ABP + + +</td>
</tr>
<tr>
<td>Saunders 1</td>
<td>N.A.</td>
<td>++ + +</td>
<td>N.A.</td>
<td>+ + +</td>
</tr>
<tr>
<td>Saunders 2</td>
<td>N.A.</td>
<td>– to ±</td>
<td>N.A.</td>
<td>+ + to + + +</td>
</tr>
<tr>
<td>Saunders 3</td>
<td>N.A.</td>
<td>–</td>
<td>N.A.</td>
<td>–</td>
</tr>
</tbody>
</table>

* Symbols used as in Table 1.
gations of fibroblasts (Plate, fig. H). As more fibroblasts condensed on the matrix area the matrix was extended. No C-S-B or polysaccharides were demonstrated in such precartilage either initially or in the final vertebral elements of the 140 mm embryo.

Table 3. The progression of AMPS in the development of Raja binoculata

<table>
<thead>
<tr>
<th>Embryonic stage</th>
<th>Neutral polysaccharides</th>
<th>Hyaluronic acid</th>
<th>C-S-A/C-S-C</th>
<th>C-S-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleavage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-4 mm</td>
<td>Cleavage furrows, general cytoplasm</td>
<td>ECM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-7 mm</td>
<td>Endoderm, mesoderm, neural ectoderm</td>
<td>ECM, mesoderm cells, neural ectoderm cells</td>
<td>Notochord sheath</td>
<td></td>
</tr>
<tr>
<td>17-18 mm</td>
<td>Notochord cells, notochord sheath</td>
<td>Notochord sheath, mesodermal basement membranes, sheath mesenchyme, trunk mesoderm</td>
<td>Notochord sheath</td>
<td></td>
</tr>
<tr>
<td>40 mm</td>
<td>Inner layer of sheath, heart mesoderm, epidermis</td>
<td>Skeletal pre-cartilage, sheath mesenchyme, trunk mesoderm, heart mesoderm, mesonephric tube cells</td>
<td>Skeletal pre-cartilage, middle layer of sheath, sheath mesenchyme</td>
<td>Middle layer of sheath</td>
</tr>
<tr>
<td>70 mm</td>
<td>—</td>
<td>Mantle neurons, gut mesoderm, heart mesoderm, mesonephric tube cells</td>
<td>Middle layer of sheath, outer layer of sheath, skeletal pre-cartilage</td>
<td>Middle layer of sheath</td>
</tr>
<tr>
<td>140 mm</td>
<td>—</td>
<td>Cartilage matrix</td>
<td>Cartilage matrix</td>
<td>Epidermis</td>
</tr>
</tbody>
</table>

DISCUSSION

Clearly, through Raja binoculata development, there occurs a progression in the appearance of AMPS (see Table 3). Neutral polysaccharides are the main constituent of cleaving cells but any gradient of polysaccharide location noted is undoubtedly due to a fixation artifact since the positioning of the polysaccharides observed in the large yolky cleaving cells suggests that displacement occurred during fixation. On the other hand, in the 2-4 mm and 6-7 mm stages,
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the gradient of neutral polysaccharides observed can be related to morphogenetic activities occurring and might possibly indicate the presence of oligosaccharide precursors for AMPS production. However, some neutral polysaccharides do persist in certain areas, e.g. heart and epidermis, until the 40 mm stage.

Hyaluronic acid, the weakly acidic AMPS occurring with neutral polysaccharides during neurulation in the form of ECM, may function as an agent of tissue adhesion, of tissue movement, or as a medium governing some aspect of the extracellular space. This material seems to possess some of the characteristics of the ‘Extracellular material’ described by Moscona (1960), though Steinberg (1963) suggested that such ECM was artificially produced from the chromosomes of cells dissociated by enzymic action. The appearance of ECM only in neurulae, and then only in specific embryonic locations, in the skate strongly suggests a natural occurrence and a specific function. Certainly, the hydrated structure of hyaluronic acid would form a diffusion barrier, a consideration which supports the possible function of ECM as a medium affecting some chemical aspect of the extracellular space. In any event, hyaluronic acid persists through development and adult stages of Raja as a component of cartilage matrix.

Chondroitin sulphates A and C participate primarily in cartilage formation and persist in adult stages as the major AMPS component of cartilage matrix. In cartilage formation AMPS appeared as a uniform matrix between closely packed fibroblasts condensed around the notochord sheath or in a place where skeletal elements were to be formed. No neutral polysaccharides were demonstrated in association with these cells either before or after AMPS formation. AMPS were not identified within the fibroblasts but only in the interstitial matrix where the density of AMPS staining seemed to depend on the proximity of the fibroblasts one to another. This is in agreement with the study of Mancini, Vilar, Stein & Fiorini (1961) which showed in cultures of fibroblasts (chick) that sulphate was incorporated into both fibroblasts and intercellular matrix but that histochemical tests for AMPS were positive only in matrix. This suggests that low molecular weight precursors are present within the cells and are polymerized to AMPS externally. The function of C-S-A and C-S-C is structural, though they may participate in some aspect of neuron development in the 70 mm embryo neural tube mantle layer as suggested by Szabo & Roboz-Einstein (1962).

Chondroitin sulphate B appears as a structural layer of the notochord and in unicellular mucous glands of the epidermis in the advanced embryo. Seno & Meyer (1963) found C-S-B to be the major AMPS of adult shark skin, and Mathews (1962) found C-S-A and C-S-C to be the major AMPS of shark cartilage.
SUMMARY

1. Histochemical treatments specific for hyaluronic acid, C-S-A/C, C-S-B and heparin were applied to a series of Pacific great skate (Raja binoculata) embryos from cleavage to immediate prehatching stages, in order to characterize histochemically the AMPS present in the embryos and to study the events leading to AMPS localization in the adult.

2. A progression was observed: (a) intracellular neutral polysaccharides in cleavage stages, (b) a combination of extracellular polysaccharides and weakly acidic AMPS (hyaluronic acid) associated with cell processes in neurulating stages, (c) extracellular strongly acidic AMPS (chondroitin sulphates) in later stages, particularly in areas of cartilage development.

3. In neurulating embryos hyaluronic acid appeared in quantity between adjacent tissues, suggestive of some developmental significance for this compound. Hyaluronic acid also appeared after neurulation in close association with developing gut and mesonephros.

4. Histochemical localization of these compounds in immediate prehatching stages agreed with previously reported biochemical analyses of shark skin and cartilage, i.e. C-S-B occurred in skin and C-S-A/C was a major component of cartilage matrix.

RÉSUMÉ

Les mucopolysaccharides acides dans le développement de la grande raie du Pacifique

1. Des traitements histochemiques spécifiques de l'acide hyaluronique, des sulfates de chondroïtine A et C (C-S-A/C), du sulfate de chondroïtine B (C-S-B) et de l'héparine ont été appliqués à une série d'embryons de la grande raie du Pacifique (Raja binoculata) à des stades allant du début de la segmentation jusqu'à ceux précédant immédiatement l'éclosion, dans le but de caractériser histochemiquement les mucopolysaccharides acides (AMPS) présents dans les embryons et d'étudier les événements conduisant à la localisation des AMPS chez l'adulte.

2. Une progression a pu être observée: (a) polysaccharides neutres intracellulaires durant la segmentation, (b) une combinaison de polysaccharides extracellulaires et d'AMPS faiblement acids (acide hyaluronique) associée aux processus cellulaires de la neurulation, (c) des AMPS extracellulaires fortement acides (sulfates de chondroïtine) dans les stades tardifs particulièrement dans les régions où se développe le cartilage.

3. Dans les embryons en cours de neurulation l'acide hyaluronique apparaît entre des tissus adjacents en quantité telle qu'elle suggère un rôle significatif de ce composé dans le développement. L'acide hyaluronique apparaît aussi après la neurulation, étroitement associé à l'intestin et au mesonephros en train de se développer.
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4. La localization histochimique de ces composés dans les stades précédant immédiatement l’éclosion est en accord avec les analyses biochimiques, rapportées précédemment, de peau et de cartilage de requin, c’est à dire le sulfate de chondroitine B se trouve dans la peau et les sulfates de chondroitine A et C sont des composants essentiels de la matrice cartilagineuse.

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REFERENCES


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