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Ultrastructural Studies on the Developing Cranial Cartilage in several species of larval fish

by

Joel E. Bodammer[1]
U.S. Department of Commerce
National Marine Fisheries Service
Northeast Fisheries Center
Oxford Laboratory
Oxford, Maryland 21654
U.S.A.

SUMMARY

Chondrocytes observed in procartilage found under the developing forebrain of striped bass (Morone saxatilis), winter flounder (Pseudopleuronectes americanus), and summer flounder (Paralichthys dentatus) larvae had two distinctly different appearances. Some cells appeared to be actively elaborating and secreting an amorphous glycoprotein-like material (presumably chondromucoprotein) which resembled the slightly more condensed substance found in the matrix that surrounded them. Others appeared to be degenerate or effete in that their cytoplasm was disrupted with large vesicles, their nuclei were condensed and heterochromatic, and in some instances their cell membranes were no longer intact. Interstitial growth of the cartilage appeared to be minimal for neither cell division nor isogenic groups of chondrocytes were readily observed. The cartilage was delimited by a well developed perichondrium with growth presumably occurring by apposition. The relevance of chondrogenesis to feeding in larval fishes and the possible effects of contaminants on normal development are considered.

RESUME

Les chondrocytes (les cellules chondroïdes) observés dans le pro-cartilage qui se trouve sous l'avant-cerveau larvaire des perches rayées (Morone saxatilis), des flets d'étés et d'hivers (Paralichthys dentatus et Pseudopleuronectes americanus) avaient deux aspects qui sont distinctement différents. L'on de la deux morphologies avait des cellules qui activement élaboraient et sécrétaient une matière amorphe et ressemblée au glycoprotéide (par présomption, un chondromucoprotéide) qui ressemblait la substance un peu plus concentrée qui se trouvait dans la matrice entourante. L'autre conformation de ces cellules se montrait dégénérée ou épuisée parce que le cytoplasma était rompu par des grosses vésicules, les noyaux étaient concentrés et hétérochromatiques et, de temps en temps, les membranes cellulaires n'étaient plus intactes. Le développement l'interstitiel se montrait minimum

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parce que souvent on n'a pas observé la division des cellules ou les groupes des cellules isogènes. Le cartilage était délimité par un périchondre et on suppose qu'un cartilage nouveau se formera par suite du développement appositional. On examinait le rapport de la chondrogenèse à la nourriture par des poissons larvaires aussi bien que les effets possibles des contaminations sur le développement normal des poissons.

INTRODUCTION

Upon depletion of their embryonic reserves, larval fish that cannot feed exogenously or obtain adequate numbers of prey starve and usually die (O'Connell, 1976). Frequently, laboratory studies of feeding and growth in fish larvae have focused their attention on numerical relationships between predator and prey (predator-prey density) and its importance to survival (see Werner and Blaxter, 1980 for references). Studies of this type are often based on the premise that laboratory-reared larvae develop normally and are as capable of feeding as those from the natural environment. Larval fish reared in captivity, however, may demonstrate skeletal (Komada, 1980) or alimentary tract abnormalities (Twongo and MacCrimmon, 1977) which may affect or preclude their ability to capture or ingest prey.

This report presents results from a series of introductory studies (Bodammer, 1979) on several species (Morone saxatilis, Pseudopleuronectes americanus, and Paralichthys dentatus) of fish larvae which are being cultured in the laboratory. The objective of this research is to examine developing cells and tissues (e.g., sensory, digestive, skeletal) that play a role in feeding. Hopefully, the results of this research will be useful in assessing the effects of larval fish culture techniques (Blaxter, 1969) and eventually those of environmental contaminants on larval development and survival.

The larvae examined in this study exhibited no deformations in skeletal structure of the head (or elsewhere), and the data presented are intended to provide insights on the status of cranial cartilage development in normal animals.

MATERIALS AND METHODS

Fish Larvae

Winter flounder and summer flounder larvae were obtained from the National Marine Fisheries Service Laboratory, Narragansett, Rhode Island, where they were hatched and reared according to techniques developed by Smigielski and Arnold (1972) and Smigielski (1975). The winter flounder larvae were sacrificed 6 days after hatching, while the summer flounder were 28 days old at the time they were preserved. Food was observed in the digestive tract of all specimens examined.

Striped bass larvae were obtained from the Edenton National Fish Hatchery, Edenton, North Carolina, where they were cultured using techniques developed at that facility (Atstupenas, pers. commun.). The larvae were raised in continuous-flow tanks at water temperatures between 17-19°C and fed brine shrimp several times daily. The specimens used were sacrificed 9 days after hatching; however, the gut contents of these fish were not examined.

Light and Electron Microscopy

The larvae were fixed for light and electron microscopy by immersing them in ice cold (0-4°C), 2% glutaraldehyde adjusted to pH 7.4 with 0.1 M phosphate buffer. After rinsing in buffer solution, they were allowed to come to room temperature and postfixed for 1 hr in osmium tetroxide in 0.1 M phosphate buffer at pH 7.4. They were then stained en bloc with aqueous uranyl acetate according to the method of Terzakis (1968), dehydrated in a graded series of ethanols and propylene oxide, infiltrated, and embedded in Spurr (1969) low viscosity medium. Thick sections (1.0-2.0 µm) were made with glass knives, stained with toluidine blue, and examined with the light microscope. Thin sections were obtained with a diamond knife and stained with alcoholic uranyl acetate and lead citrate before being examined with a Zeiss EM 9 S2 electron microscope.

RESULTS AND DISCUSSION

In winter flounder larvae (Fig. 1), the cartilagenous plate lying dorsal to the oral chamber (OC, Fig. 1) contained a number of oval-shaped chondrocytes lying in close proximity to each other as is typical for procartilage. The nuclei of the cells were round and of variable staining intensity. The cytoplasm of the chondrocytes varied from a uniform light blue to a darkly stained, condensed condition accompanied by large vacuoles. The chondrocytes were not in pairs or clusters (isogenic groups), nor was the capsule and lacuna normally surrounding these cells in hyaline type cartilage observed. Except for its width, which was larger in striped bass and summer flounder larvae, the cartilagenous plate occupied the same anatomical position in all three species. In summer flounder larvae, however, more chondrocytes appeared to have the condensed and vacuolated cytoplasm than was observed for the other species.

Ultrastructurally, the chondrocytes in winter flounder larvae (Figs. 2, 3) procartilage demonstrated several different morphological types which are presumed to be related to their age and secretory activity. In Figure 2, a putative effete cell (EC) is shown near the periphery of the cartilage. Cells of this type routinely had a disrupted cytoplasm containing many large empty spaces--hence their vacuolar appearance with the light microscope. A mature secretory cell (SC, Fig. 2) is present next to the effete cell, and its cytoplasm is filled with vesicles which contained a moderately dense material that resembled the nonfibrillar, amorphous substance seen in the adjacent matrix (MA, Fig. 2). Somewhat younger appearing secretory cells (Fig. 3) also contained numerous vesicles; however, the stainable material within them was less condensed than in the vesicles of the mature chondrocytes. Their nuclei demonstrated a euchromatic appearance suggesting ongoing genetic activity (note contrast with effete cell shown in Fig. 2). The mitochondria of these cells were quite large and had tubular cristae (M, Fig. 3). The Golgi complex, however, was not particularly noticeable and consisted of only a few flattened saccules and very small vesicles.

The electron microscopic appearance of the chondrocytes was similar in all the larval fish species examined. Perhaps owing to their age, greater numbers of degenerate cells appeared to be present in the procartilage plate of striped bass and summer flounder larvae (Fig. 4). These older larvae, however, proved

to be more suitable specimens for possible observations of cartilage growth by apposition in which new chondrocytes are added along the periphery of the perichondrium (PC, Fig. 5). This type of growth allows for the distribution of material in a manner such that the appropriate form and size of the cartilage is attained (Bélanger, 1977).

The causes of skeletal abnormalities involving the head and jaws of larval fishes are incompletely understood (Rosenthal and Alderdice, 1976). As Komada (1980) has shown, these anomalies may be much more prevalent in cultured fish compared to those captured from their natural environment. Because laboratory-raised larval fish are often used in various studies on growth and survival (e.g., feeding energetics, physiology, pollution effects), we are beginning our research by studying the normal morphology of cells and tissues that are involved in feeding and which may be affected by either laboratory conditions or environmental pollutants (Mehrle and Mayer, 1977; Couch et al., 1979; Ozoh, 1980).

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FIGURE LEGENDS

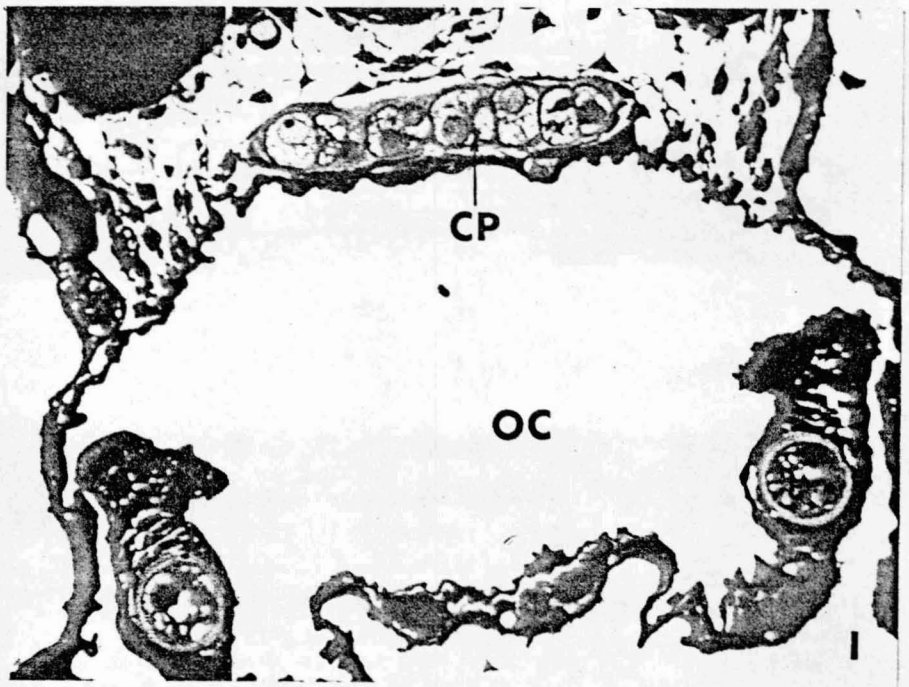
Fig. 1. Light micrograph of a winter flounder larvae in cross-section illustrating chondrocytes within the procartilage plate (CP) near the dorsal aspect of the oral cavity (OC). X693.

Fig. 2. Electron micrograph of chondrocytes from winter flounder larvae featuring an effete cell (EC) adjacent to an active secretory cell (SC). (OM = oral mucosa; MA = cartilage matrix; N = cell nucleus). X7,220.

Fig. 3. Electron micrograph of developing chondrocytes in winter flounder procartilage. (N = cell nucleus; M = mitochondrion). X7,220.

Fig. 4. Electron micrograph of two effete cells (EC) observed in the procartilage of a striped bass larvae. X7,220.

Fig. 5. Electron micrograph of the procartilage from a striped bass larvae illustrating a putative developing cell (DC) located near the perichondrial cell layer (PC). X7,220.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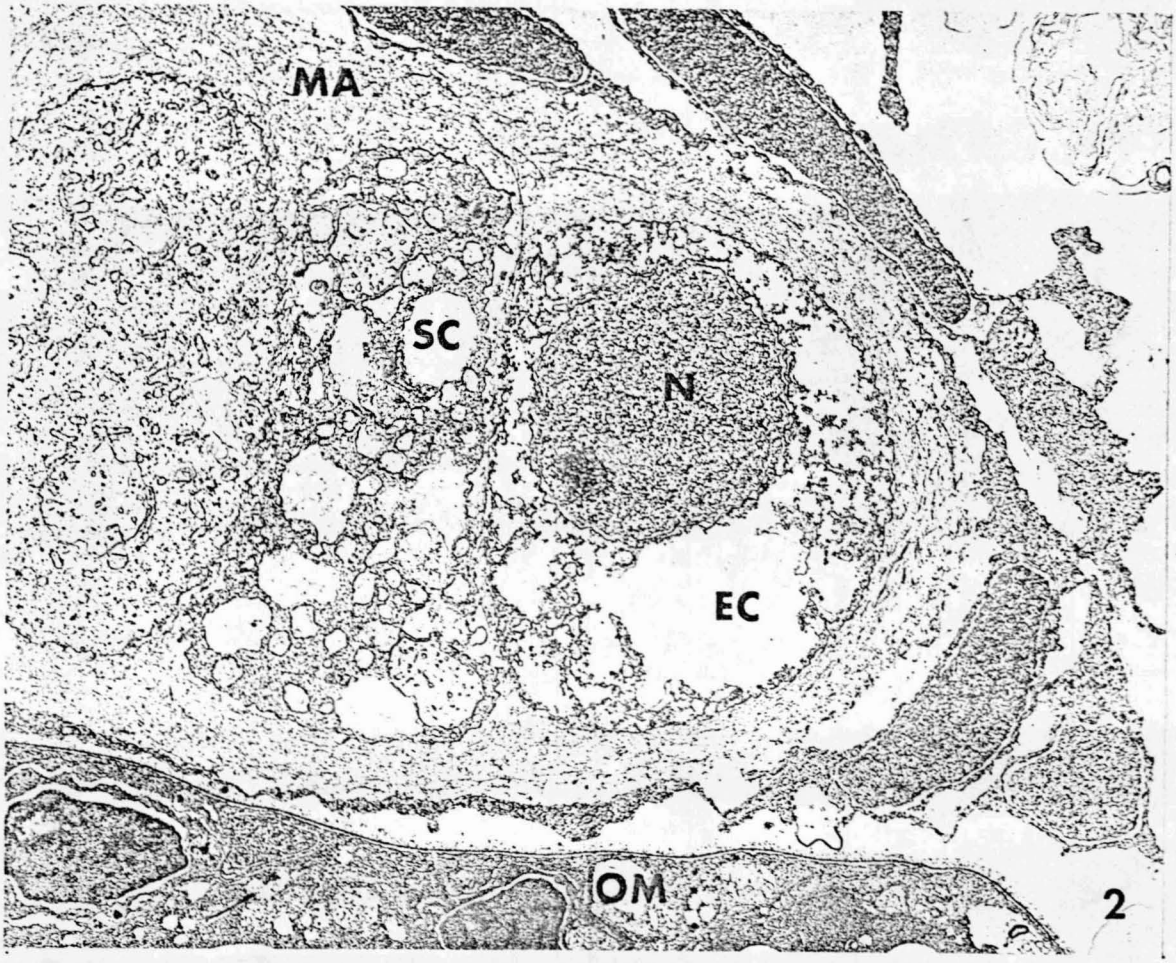
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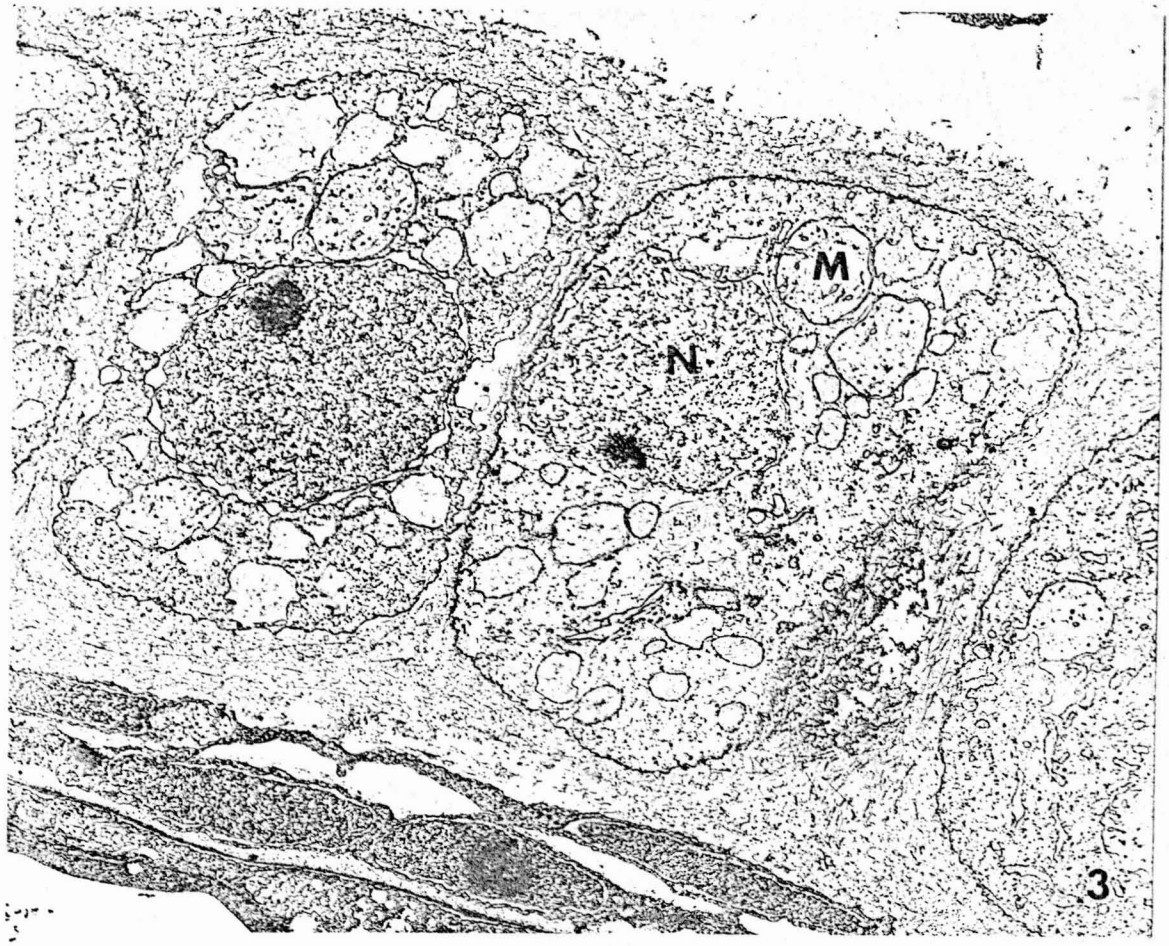
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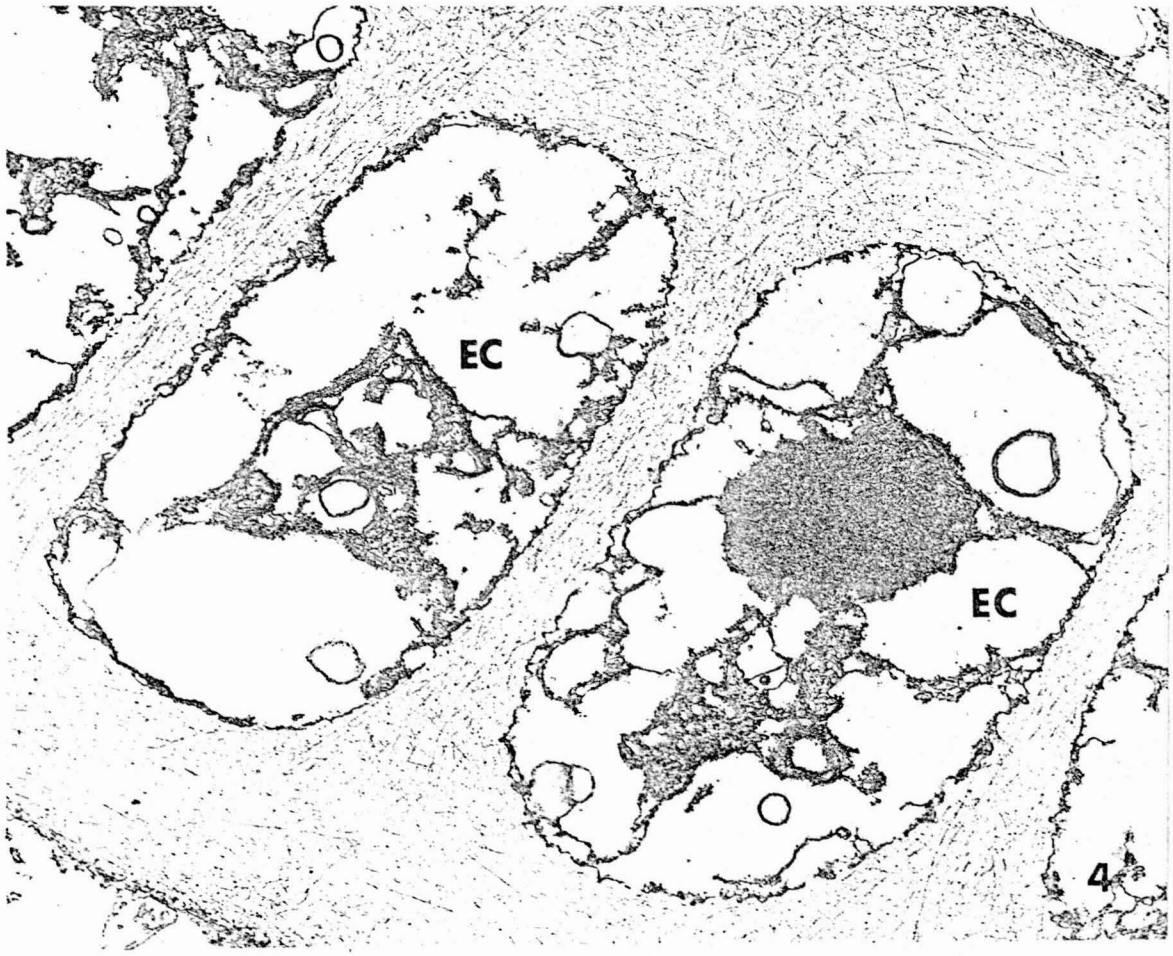
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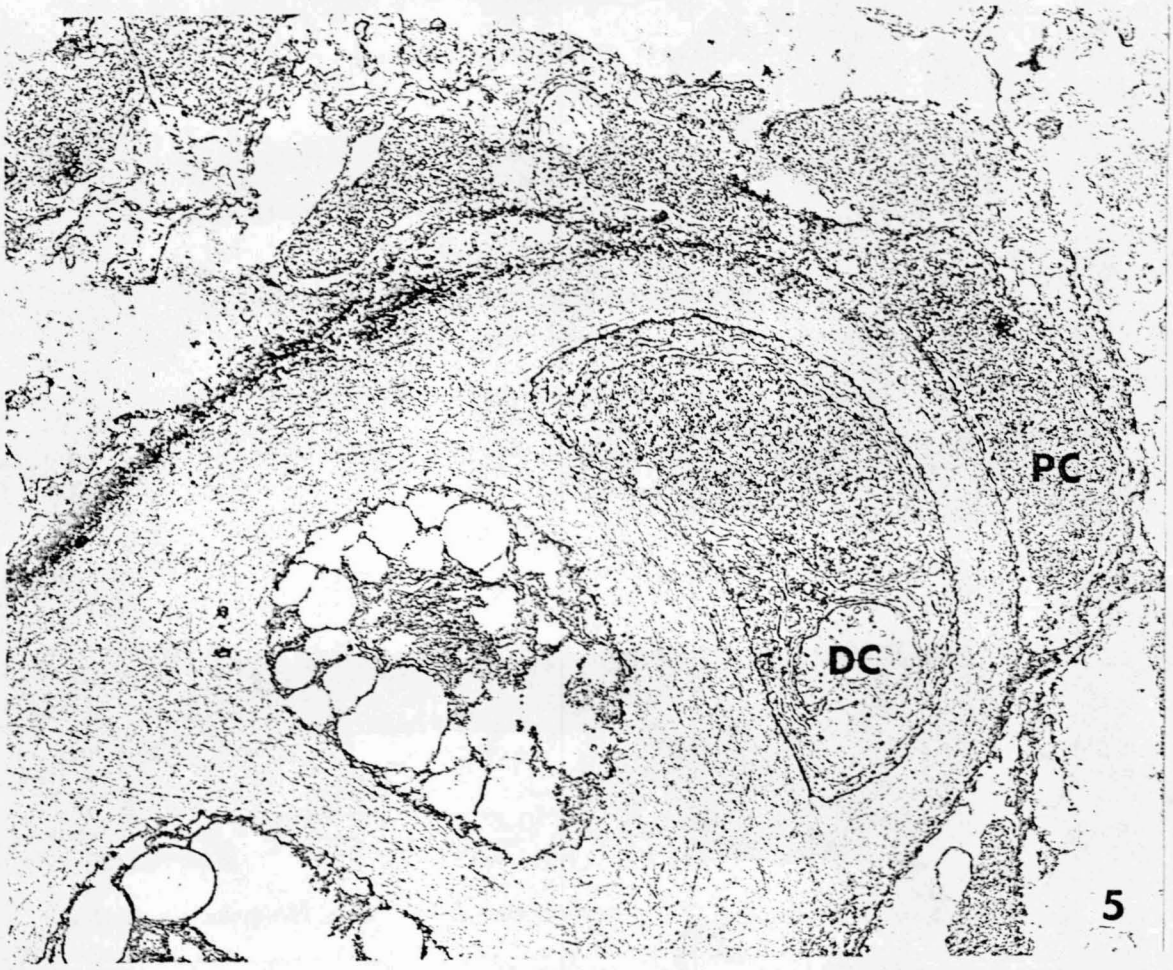
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