Observations Concerning The Non Mineralized Skeletal Components Of E. parma.

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In a previous report it was suggested that the fibrous component of the skeletal system was elaborated by primary and (possibly) secondary mesenchyme cells. In subsequent studies this concept has been modified. Our observations indicate that a number of delicate fibers and fiber systems are elaborated before the skeleton is fabricated. These fibers appear to be formed by small stellate cells reminiscent of fibroblasts in in higher organisms.

In the skeleton these structures have the appearance of collagen fibers. They may be demonstrated by treatment with Mallory Azan but not by methods used to demonstrate reticular tissue. Under polarized light they reveal birefringence indicative of molecules oriented in a linear arrangement. Treatment with toluidine blue indicates that they are orthochromatic, and faintly PAS positive. The ground substance in which these fibers lie was shown by histochemical methods to contain a very soluble PAS (muco polysaccharide) component. This component could not be demonstrated by conventional PAS methods, but only after periods of combined precipitation and decalcification proceedures. The organic components of the skeleton of E. *parma* consists of a fibrous collagen-like component and a ground substance containing a muco polysaccharide. The structure and composition of these substances though similar in some respects differs in detail from analogous structures in higher organisms.

Inhibition Of Calcification In Regenerating Teleost Scales Following Administration Of Tetracycline

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Adult *Fundulus heteroclitus,* weighing an average of approximately 5 grams each, were given daily intraperitoneal injections of 0.1 mg tetracycline in 0.1 cc distilled water. Control fishes were injected with equal amounts of distilled water. Scales were plucked from the left flanks of all fishes at the onset of injections, and the animals were maintained in running salt water aquaria at 20-22°C. After three weeks, the fish were sacrificed and their scales stained for calcium with the von Kossa technique.

Regenerated scales of control fishes had acquired several growth rings and were heavily calcified. Scale regenerates from tetracycline treated fishes were extremely flaccid in contrast with the stiffened control regenerates, and exhibited considerably less calcification. Calcium deposition

had occurred lightly and homogeneously over the large central areas of the regenerated scales, while in the peripheral areas it was limited to the outer margins of the concentric growth rings. Calcification was heaviest in the oldest, innermost growth ring; it decreased with successively younger rings and was absent around the outermost margin of the scale.

By virtue of the fluorescent nature of tetracycline, its location in the scales of experimental fishes could be determined by examination under ultra violet light. In regenerated scales, this revealed a pattern of fluorescence exactly coincident with that of calcification described above. In normal scales from tetracycline treated fishes, fluorescence was limited to the very peripheral regions, being especially concentrated on the anterior and lateral margins, and almost absent from the posterior edge of the scale. These results indicate that at the doses employed, tetracycline inhibits calcification of regenerating teleost scales, but is itself localized wherever the limited amounts of calcium are deposited. This spatial correlation between tetracycline localization and sites of calcification testifies to the probability of a direct effect of the former upon the latter. Despite deficient calcification, however, the growth rates of regenerating scales did not appear to be seriously retarded.

Inhibition Of Mineralization In The Embryo Of E. parma And The Mangrove Oyster

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Our previous studies show that embryos of E. *parma* which are reared in sea water containing tetracycline develop skeletons which may be partly or completely inhibited. In an attempt to assess the action of this drug on the mechanism of mineralization, the Ca^{++} and Mg^{++} content of the sea water was increased in excess of the theoretical complexing power of tetracycline. Although these ions were present in sea water much in excess of the normal amounts, protection against inhibition of mineralization did not occur. Examination of the tri-radiate crystal, the skeletal anlage, shows that this structure, a calcite crystal, is either completely absent, or much reduced in size when subjected to the drug environment during development. Similar experiments conducted on the mangrove oyster (Pedalion) show that tetracycline is incorporated in the regenerating and newly formed shell; that the amount and degree of mineralization is reduced, and in addition, the size of the calcite crystals are reduced in size by this treatment. From the above observations it appears that tetracycline inhibition of mineralization consists first in complexing with the calcium and possibly other cations which subsequently results in the inhibition or modfiication of the orderly crystal growth observed in normal specimens.