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EXTRACELLULAR MATRIX: FUNCTIONAL SIGNIFICANCE OF OXYTALAN,ELAUNIN AND ELASTIC FIBERS

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INTRODUCTION

Much attention has been paid to the extracellular complex of macromolecules that includes collagens, elastin, proteoglycans and glycoproteins. They influence the cell behaviour in general during development and adult life and are sources of strength,resilience and cohesiveness to the tissues (Hay,1981).

Significant advances have contributed for elucidating many aspects of the nature of different constituents of extracellular matrices (Deyl and Adam,1981; Hukins,1984; Reddi,1985; Mecham,1986), but little is known about the composition and functional significance of the tissue structures that contain elastin.Elastic fibers are responsible for most of the elastic properties of many vertebrate tissues. These fibers have been described by Ross and Bornstein(1969) as consisting of an amorphous core of elastin surrounded by microfibrils of 10-12 nm in diameter. Other elastic-related fibers (oxytalan and elaunin fibers) have been visualized as formed by bundles of elastic microfibrils (oxytalan) or microfibrils intermingled with patches of amorphous elastin (elaunin) (Fullmer and Lillie,1958; Gawlik,1965; Cotta-Pereira et al.,1975, 1976a,1977).

The dermal elastic system fibers described by Cotta-Pereira and colleagues(1976a,1978) and confirmed by Schwartz and Fleischmajer(1986) as including oxytalan,elaunin and elastic fibers represent a useful model for studying the interaction between microfibrils and amorphous elastin during the maturation of elastic fibers.The work of Schwartz and Fleischmajer (1986)also suggests that oxytalan fibers may represent the initial step of elastogenesis. However, there are several other tissues where the study of elastic system fibers may help to clarify the nature and functional significance of those extracellular components.In this respect, the morphology of ciliary zonule and the annular ligament in rats were studied using light and electron microscopy. Also, aortae of chick embryos and adult unvertebrates (annelids, molluscs and crustaceans) were studied in the same way in order to show some aspects of ontogenesis and phylogenesis of elastic fibers.

MATERIAL AND METHODS

Fragments of eyeballs and temporal bones of young adult rats were fixed in 10% formaldehyde and embedded in paraffin. Other fragments were fixed in 3% glutaraldehyde dissolved in 0.1 Millonig buffer (pH=7.3) containing 0.25% of tannic acid, post-fixed in 1% osmium tetroxide and embedded in Epon (Cotta-Pereira, 1976b). Temporal bones were previously decalcified before embedding. Also fragments of chick embryos from the 24th until 36th stage (Hamburger and Hamilton, 1951) and adult earthworms (P. hawayana), bivalve mollusc (P. perna) and river shrimp (M. carcinus) were fixed both for light and electron microscopy.

The paraffin sections for light microscopic observations were stained with haematoxylin and eosin, iron haematoxylin (Verhoeff, 1908) and resorcin-fuchsin (Weigert, 1898). While Verhoeff's iron haematoxylin demonstrates fully mature elastic fibers selectively, elaunin and oxytalan fibers are not stained by this method. Weigert's resorcin-fuchsin selectively stains both elastic and elaunin fibers, whereas oxytalan fibers remain unstained when they are not previously oxidized. Oxidation was performed using oxone as previously described (Fullmer et al., 1974).

RESULTS

Ciliary zonule. All fragments obtained from eyeballs of rats showed the ciliary zonule as formed by fibers that stained selectively for oxytalan fiber. At the electron microscope they disclosed typical pattern of oxytalan fibers (bundles of microfibrils of 10-12 nm in diameter, with a tubular appearance) (Figure 1).

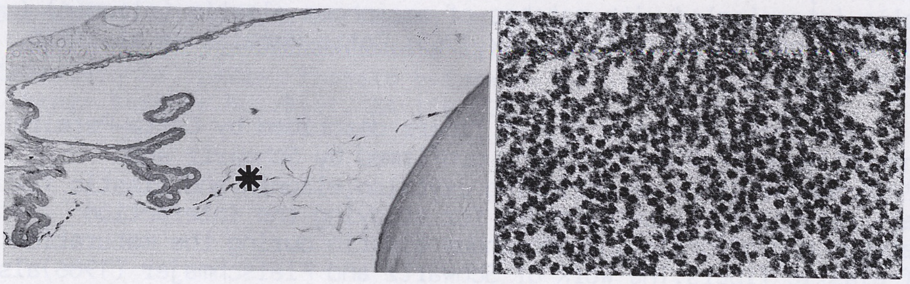

Figure 1. Left: Photomicrograph of ciliary zonule (asterisk) binding the lens to the ciliary body. Resorcin-fuchsin after oxidation, x200 Right: Electron micrograph of a zonular fiber showing cross sectioned microfibrils. Tannic acid fization, x80,000.

Annular ligament. The articulation between the base of the stapes and the margin of the oval window showed fibers with tinctorial and ultrastructural characteristics of elaunin fibers,anchoring into both articular surfaces as oxytalan fibers (Figure 2).

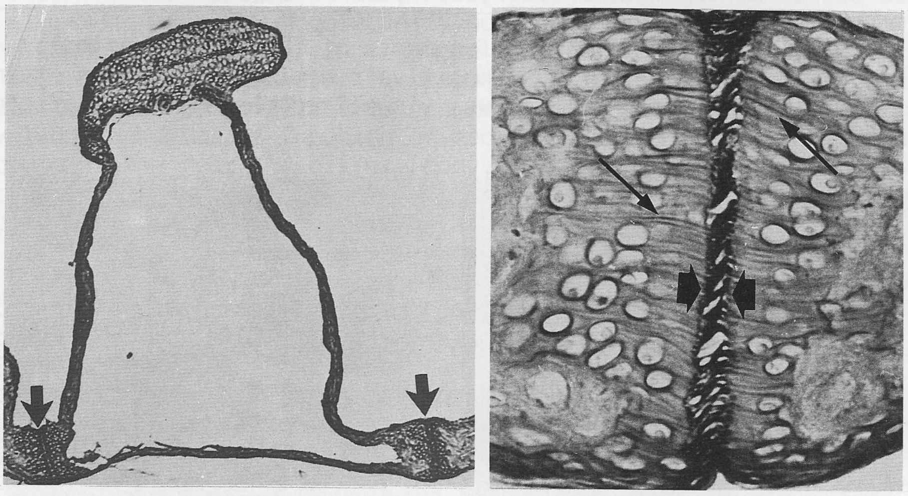

Figure 2. Left:Photomicrograph of the stapes and its articulation (arrows)with the oval window.H-E stain, x300. Right:Photomicrograph of the annular ligament showing elaunin fibers binding both articular surfaces(thick arrows)and oxytalan fibers(thin arrows)embedded into the cartilages.Resorcin-fuchsin after oxidation, x1,500.

Chick embryo aortae. Under the light microscope,the transverse sections of chick embryos have shown the presence of oxytalan fibers in the wall of developing aortae during the 29th stage,while elaunin fibers were visualized after 34th stage and elastic ones only after 36th stage. At the electron microscope patterns of oxytalan,elaunin and elastic fibers were coincident with the light microscopic observations (Figure 3).

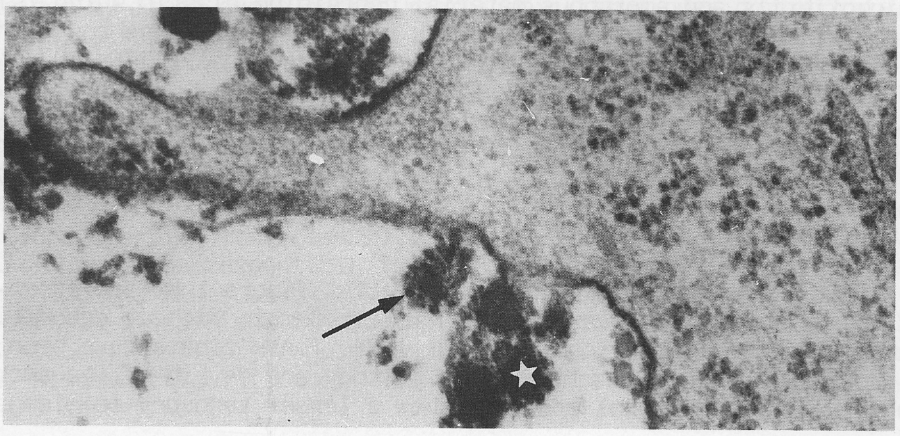

Figure 3.Electron micrograph of the wall of a dorsal aorta at the 34th stage (8th day of incubation)in the chick embryo. Observe microfibrils (arrow) intermingled with scarce dense amorphous material (star) in the extracellular matrix sinthesized by a smooth muscle cell. Tannic acid fixation, x 60,000.

Unvertebrate aortae. The fragments of the studied animals showed, in paraffin sections, positive reaction to oxytalan stains. At the electron microscope it was visualized the oxytalan pattern. (Figure 4).

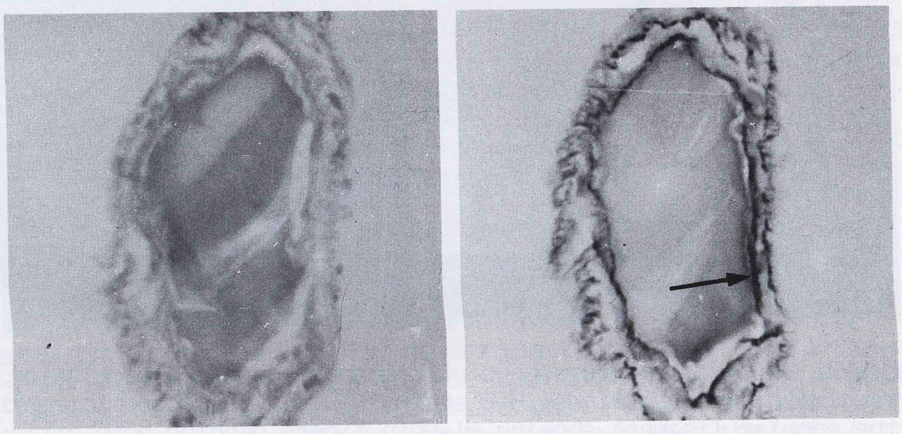

Figure 4. Left: Photomicrograph of dorsal artery of P.hawayana(an earthworm)stained with resorcin-fuchsin without previous oxidation. We don't observe any fiber . x 450. Right: Photomicrograph of the same artery stained with resorcin-fuchsin after oxidation. Observe oxytalan fibers in the artery wall(arrow), x 400.

DISCUSSION AND CONCLUSIONS

The present concept on the chemical composition of elastic fibers is basically that one appointed by Ross and Bornstein (1969): microfibrils and amorphous material containing elastin,which is responsible for the elastic properties of such fibers(Ross and Bornstein,1969; Robert et al.,1971; Cotta-Pereira et al.,1976a,1977; Robert and Robert,1980)

During the elastic fiber formation, the microfibrils appear first, followed by the amorphous component, which is gradually deposited between the m icrofibrils until the fiber is full matured (Fahrenbach et al.,1966).

However, in certain anatomical sites,fibers that are essentially formed by bundles of microfibrils remain without depositing amorphous elastin during the adult life. These fibers have been identified as oxytalan fibers and if they are devoid of the amorphous elastic material probably show a lesser tendency to elongate under mechanical stress (Cotta-Pereira et al.,1976a,1977).This hypothesis is consistent with the fact that oxytalan fibers have been found in locations where resistance to mechanical stress is required,such as periodontium (Fullmer and Lillie,1958),dermoepidermal junction (Cotta-Pereira et al.,1976a,1978),cartilage (Cotta-Pereira et al.,1984) and, as unequivocally now demonstrated,in the ciliary zonule. Our findings corroborate the observation of cross-reactivity between zonules and elastic tissue microfibrils by immunocitochemistry(Streeten,1982).It is possible that the zonular fibers are involved in a similar function of mechanical resistance; these fibers,departing from the ciliary body,reach the lens capsule and act by supporting the strength of the ciliary muscle upon the lens during the visual accommodation.

Elaunin fibers,containing microfibrils and a little amorphous material is expected to display elastic properties intermediate between those of elastic and oxytalan fibers (Cotta-Pereira et al., 1975,1976a,1977).As a matter of fact elaunin fibers may have an important role in the articulation between the base of the stapes and the margin of the oval window (fenestra vestibuli) because they predominate in this so-called annular ligament and are continuous with oxytalan fibers which are embedded in the matrix of the articular cartilage. Although the functional implications of these morphologic findings are a matter of speculation,the absence of elastic fibers and the presence of elaunin and oxytalan fibers instead suggest that a modulation of elasticity is required by this particular articulation.

Finally, it appear expedient to study the mechanism of elastogenesis both ontogenetically and phylogenetically.

During the formation of the chick aorta,mesenchymal cells of the wall of such developing arteries synthesize bundles of microfibrils which stain as oxytalan fibers.These fibers change the stainability to elaunin (34th stage) and elastic fibers (36th stage), when elastin is deposited between microfibrils. These findings , obtained by light and electron microscopic techniques corroborate a former observation of Gawlik(1965) who suggested the sequence: oxytalan-elaunin-elastic fibers after studying human fetuses. Recently, Schwartz and Fleischmajer (1986) also demonstrated,after studying the dermal elastic system fibers, by immunocitochemistry, that oxytalan,elaunin and elastic fibers constitute a sequence during the elastogenetic process.

One question, that remains to be answered , refers to which factors would be responsible for the interruption of elastogenesis remaining the fiber as oxytalan or elaunin during the adult life, in certain tissues and disclosing relevant functions of mechanical resistance in ciliary zonule,modulate elasticity in the stapes-oval window articulation or, when mature,improving full elasticity to the artery wall?

Probably lesser complex organisms, like unvertebrates,could led us to any speculation. The artery wall of the unvertebrates studied in the present work, is formed only by oxytalan fibers,identified by both light and electron microscopy. It is reasonable to assume that the simpleness of these animals doesn't require mechanisms of elasticity in their circulatory system. As a matter of fact unvertebrates doesn't possess cross-links as detected by Sage (1982) only in vertebrate aortae after using biochemical methods. Such findings corroborate our observations on the presence of oxytalan fibers and absence of elastic fibers in unvertebrate arteries

If in lower animals only the initial steps of elastogenesis are observed, it can be speculated that this is another example of "ontogenesis recapitulating phylogenesis" (Häeckel,1874).

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