# THE ULTRASTRUCTURE OF ELASTIC FIBERS

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In the last few years, electron microscope studies have shown that elastic fibers are made up of two constituents: amorphous substance (elastin), and elastic microfibrils which have different morphology and a specific affinity for contrast substance. The latter quality is conditioned by definitely proved chemical composition distinctions (Bodley, Wood — 1972; Ross — 1973). There are conjectures, as well as partial evidence of differences in their elastic properties also. The peculiarities referred to explain the great interest in the concrete study of the elastic fibers' ultrastructure in various organs inasmuch as it reflects their functional and construction specificity. Hence, in the present study on elastic structures from the rat auricular concha and skin, we made it our aim to investigate their ultramorphology, spatial organization and fine structure interrelations.

## Material and Method

Cartilage from the auricular concha and tail skin of 10 grown up white rats (Wistar line) were studied. After killing the animals, small pieces of the respective objects were obtained and fixed in 3% glutaraldehyde (0.1 m phosphate buffer, pH 7.4) for 2 hours at temperature 4° C. and then subjected to postfixation in 1% osmium tetroxide (0.1 m phosphate buffer, pH 7.4) for 90 min at the same temperature. Dehydration: alcohol and acetone. Tissue specimen flat embedding in Durcupan ACM (Fluca). The semithin sections (0.5—1 μm) were stained with toluidine blue and pyronine, the ultrathin sections were stained with uranyl acetate and lead citrate. JEM 7A electron microscope was used in the study.

## Results

In the auricular concha cartilage the elastic structures form a spatial network, built up of linked between each other partitions (septa) and single (free) fibers. In this fashion the chondrocytes appear to be incorporated individually or in groups, within cavities of elastic material (Geyer, Tews — 1971). The elastic septa, respectively fibers, usually display equal structure regardless of their size. They are made up of a central core with amorphous appearance and peripherally situated microfibrils (Fig. 1). As a rule, the amorphous part (AP) is stained less intensely as compared to microfibrils (MF). The intensity of contrast staining at the septa and thick fibers' level is not uniform: along the section surface zones of weak density alternate with strips of medium density. In all likelihood, these areas mark the junctions between the smaller fibers which build them up through merging. AP of the elastic septa has varying thickness. In some places it is absent and intervening spaces

are produced. Most frequently the microfibrils (MF) are situated within these spaces. MF submerged in the AP are also discovered. In the peripheral parts of the cartilage the elastic partitions are thinner and possess a greater

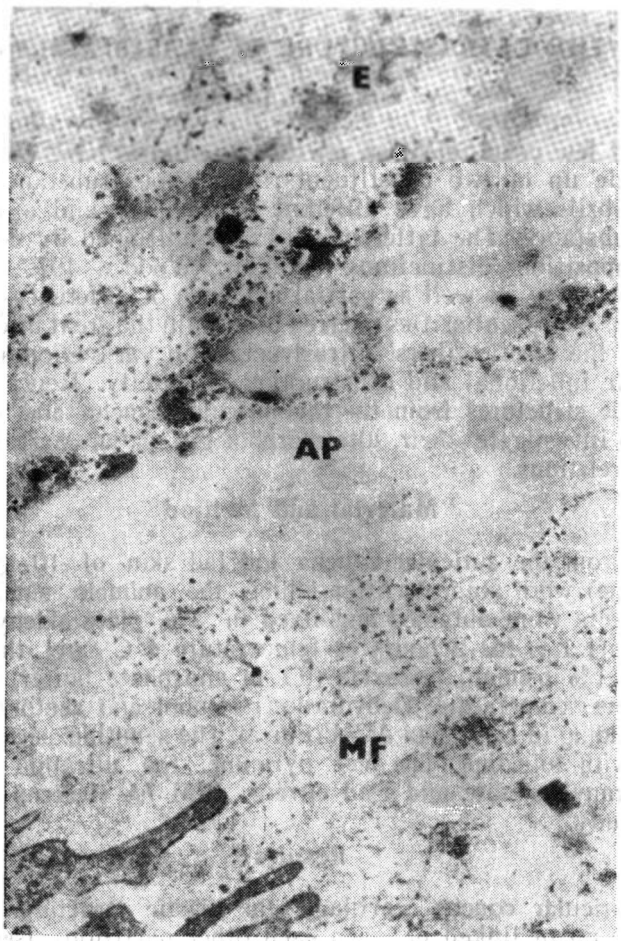


Fig. 1. Cartilage of auricular concha. AP — amorphous part of the elastic septum. *MF* — microfibrils forming a spatial network. *E* — free elastic fibers. In the left lower angle — chondrocyte. Magnif. 20,000

number of intervening spaces. This gives them the appearance of incompletely organized structures. The observed incomplete structures and slits without any doubt, contribute to the possibility of substance information exchange between chondrocytes.

Elastic microfibrils are an invariably present component of the elastic fibers and septa. However, within the cartilage they are not situated merely along the periphery of the fiber but rather fill out the entire space between chondrocytes and AP of the fibers and septa. (Fig. 1) Not infrequently they

establish contacts with chondrocytes. In rather mature chondrocytes located in the inner part of the cartilage, the zone occupied by microfibrils becomes narrower as the result of the increasing cell sizes, on the one hand, and at the

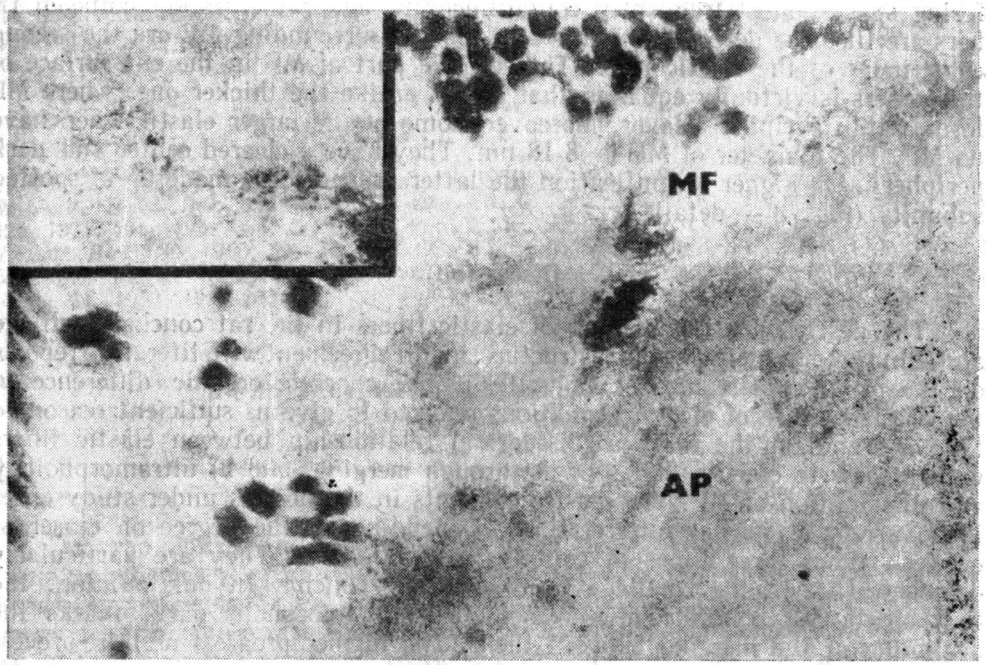


Fig. 2. Rat skin — reticullar layer. *AP* — amorphous part of a thick elastic fiber. *MF* — peripheral elastic microfibrils. Magnif. 30,000.
Detail: Transversely cut elastic microfibrils. Magnif. 35,000

expense of the thickened AP in the elastic septum, on the other. Dense contacts between chondrocytes and AP of the elastic partitions, resp. fibers, deprived of proper microfibrils, are also observed. In the periphery of the microfibrillary zone isolated, independent elastic fibers occur with growing diameter, touching each other near the septa, and becoming merged to form larger ones. In addition, some of the free fibers also form contacts with the septa. In either case of contact MF show a reduction and accordingly disappear depending on the degree of closeness. That is why contacts are observed with single MF between the merging AP, contacts deprived of MF but with a clear-cut, intensely staining junction, and contacts where the junction is marked by an amorphous band of medium density alone (Fig. 1). The MF in the cartilage have diameter 8-12 nm. Some of them are with tubular appearance not described in the pertinent literature heretofore.

In the skin the elastic fibers display an uneven pattern of distribution. They are single and thin in the papillary layer, whilst in the reticular layer their quantity increases. Here fibers with most variable diameter are established. From fine-structure viewpoint, and regardless of their size and topography, the fibers exhibit an equal build up as a rule (Fig. 2). The centrally situated AP (elastin) is much larger and has irregular shape. Against the background

of its generally weak contrast, a darker peripheral margin is clearly outlined. Along with that, in the innermost part of the AP, similarly to the cartilage, irregular in form and varying in size, unclearly demarcated areas of medium density are observed. This accounts for the spotted pattern of the AP. Interfering spaces, most frequently containing MF, and rather rarely collagen fibers are likewise detected. In this case too the surrounding MF are the second constituent of the elastic fiber. The relative part of MF in the cut surface of thin fibers is virtually equal to that of AP unlike the thicker ones where MF form a thin peripheral layer. Moreover, some of the larger elastic fibers have no MF. The diameter of MF is 8-18 nm. They have a cleared center and dark periphery. On higher magnification the latter proved to be made up of spotted subunits (Fig. 3 — detail).

## Discussion

The described ultrastructure of elastic fibers in the rat conchal cartilage and skin shows principally equal structure, in full agreement with literature reports on the problem (Greenlee et al — 1966). The concrete organic difference in the ultrastructure of elastic formations referred to give us sufficient reason to take a stand on the following issues: a) relationship between elastic fibers with a view to clarify their growth through merging, and b) ultramorphology and distribution of MF. The elastic elements in the objects under study enter in a variety of spatial interrelations. Depending on the degree of closeness between them three types of contacts can be found. They are particularly demonstrative in the cartilage from auricular concha. In our opinion, the first single MF contact between neighbouring amorphous parts marks the beginning of the merging between them. The further progress of this process leads to a gradual disappearance of MF which are being replaced along the contact line by an intensely stained junction strip with agranular appearance. At the end of merging, the junction line is once again represented by an amorphous band of medium density which conditions the spotted pattern of the thick fibers' AP (septae in the cartilage) in either of the objects. Therefore the elastic fibers in the specimens under examination might be classified not merely according to size (small and large), but also according to composition: a) simple, single, with homogeneous elastin, and b) complex, with spotted amorphous matrix, made up of merged individual fibers. The way of size increase through merging described in the elastic fibers by no means rules out their growth through elastin apposition (see below).

The microfibrils in both specimens are with practically equal ultramorphology in accordance with the description given by Low (1962) in other organs. Around the small, thin elastic fibers a strong concentration of MF is observed; in some places the part occupied by MF in the diameter of fibers is even greater than that of AP. Parallel to the small elastic fibers' growth to a certain size, most probably taking place in the beginning through elastin deposition over a network of MF (Kádár — 1974), their quantity shows a visible reduction. Some of the fibers have no MF (Albert — 1972). After the possibilities of increasing the sizes in the manner described have decreased, or other factors (e. g. local-mechanical) have imposed a quick secondary thickening of elastic fibers, the growth is accomplished through merging. A characteristic structural feature of MF in the cartilage is the fact that in addition to becom

ing merged with the elastic fibers and partitions, they form also a spatial network around the chondrocytes, and establish partial contacts with them. In all likelihood, such an organization of the MF meets the requirement to fix the chondrocyte in space by means of a microfibrillary skeleton which plays the role of a fine buffer.

## REFERENCES

1. Albert, E. N. *Amer. J. Pathol.*, 69, 1972, 89—94. — 2. Bodley, A. D., R. J. Wood. *Anat. Rec.*, 172, 1972, 71—88. — 3. Geyer, G., K. Tews. *Anat. Anz.*, 128, 1971, 491—496. — 4. Greenlee, T. K. Jr., R. Ross, J. L. Hartman. *J. Cell. Biol.*, 30, 1966, 59—71. — 5. Kadar, A. *Path. Europ.*, 9, 1974, 133—146. — 6. Low, F. N. *Anat. Rec.*, 142, 1962, 131—138. — 7. Ross, R. *J. Histochem. Cytochem.*, 21, 1973, 199—208.

## ОБ УЛЬТРАСТРУКТУРЕ ЭЛАСТИЧЕСКИХ ВОЛОКОН

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## РЕЗЮМЕ

Электронномикроскопически исследованы эластические элементы хряща ушной раковины и кожи хвоста взрослых крыс. В обоих объектах они показывают принципиально одинаковое устройство: центральная аморфная часть и периферическая, состоящая из эластических микрофибрилл. В ушной раковине микрофибриллярные элементы установлены вне эластических волокон; они формируют около каждого хондроцита зону различной ширины. В ней располагаются и единичные эластические волокна, толщина которых наростает к периферии. Эластические волокна кожи неравномерно разпределены; в глубоких слоях они толще и образуют густую сеть. Описывают различные виды контактов между эластическими волокнами обоих объектов, которые интерпретируют как фазы слияния между ними.