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Elastofibroma

A Correlated Light and Electron Microscopic Study

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Summary. Four cases of elastofibroma located in the subscapular region of 3 men aged 66, 74, and 83 years, and a woman 70 years old are reported. A correlated light and electron microscopic study including ultrastructural examination of Verhoeff's iron-hematoxylin (VIH)-stained sections was performed. Light microscopically, the elastofibromas were characterized by connective tissue built up by collagen fibers and sclerotic masses mingled with numerous fibers and globules of elastin material. In one micron thick Epon sections these elastin fibers often revealed a central axis surrounded by a mantle composed of periodic segments giving them a necklace-like appearance. The ultrastructural findings of these elastin structures, stained with VIH, and the appearance of the stroma cells and their relation to the elastin indicate that elastofibroma is a non-neoplastic reactive lesion in which elastin material is synthesized by the stromal cells and predominantly laid down around preexisting elastic fibers.

Key words: Elastin – Elastofibroma – Histochemistry – Soft tissue tumor – Ultrastructure

Elastofibroma was first described by Järvi and Saxén in 1959 at the XII:th Congress of Scandinavian Pathologists. Four tumors reported then were all located in the subscapular region and were microscopically composed of collagen, fat and a large number of granularly degenerated coarse bands and fibers with the staining characteristics of elastic tissue. Since the first report several describtions of mostly single cases or of a small series have appeared in the literature. The further studies by Järvi and coworkers led them to conclude that elastofibroma is not a true neoplasm, but rather a degenerative pseudotumor of elastic tissue (Järvi et al. 1969; Järvi and Länsimies 1975). However, the origin and the nature of the elastin staining material have been debated. Stem-

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merman and Stout (1962) suggested that the fibers in the tumor might have resulted from elastotic degeneration of collagen and that consequently the tumor should not be regarded as a proper tumor of elastic tissue. This opinion has been supported by other authors (Barr 1966).

There are few electron microscopic studies performed on elastofibroma; the observations in the previously studied four cases have not unequivocally solved the question of the origin of the elastin material (Banfield and Lee 1968; Waisman and Smith 1968; Järvi et al. 1969; Winkelmann and Sams 1969; Akhtar and Miller 1977).

Staining of thin sections with uranyl acetate followed by lead citrate does not impart electron opacity to the dense component of elastic fibers. Staining of epoxy thin sections with Verhoeff's iron-hematoxylin mixture (VIH) however has been found to impart selective electron opacity to the elastic fibers of connective tissues (Brissie et al. 1975; Spicer et al. 1975). This method seems to have several advantages over the metalloporphyrin sulphonate (Albert and Fleischer 1970; Albert 1972 and 1973) and phosphotungstic acid methods (Pease and Molinari 1960; Pease and Paule 1960; Paule 1963; Greenlee et al. 1966; Weisman and Carnes 1967; Albert and Pease 1968; Kadar et al. 1971).

The present report of 4 elastofibromas includes a correlated light and electron microscopic study. Ultrastructural examination of VIH-stained sections gives new information about the nature and origin of the elastic tissue of the lesion.

Material and Methods

The material for the study comprised the clinical histories, operative specimens, formalin-fixed tumor tissue, tissue blocks, routine histological sections, 1 micron thick sections of Epon-embedded tumor tissue and ultrathin sections for electron microscopy.

Five micron thick sections of formalin-fixed and paraffin-embedded tumor tissue were stained with the hematoxylin–van Gieson sequence and hematoxylin and eosin. Gordon's and Sweet's silver impregnation was used for demonstration of reticulin fibers and Verhoeff's iron hematoxylin (VIH), aldehyde-fuchsin, and resorcin-fuchsin were employed for the demonstration of elastic tissue.

For electron microscopy small pieces of the tumor were immediately put into 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 and 4° C. Specimens were fixed for 4 h, then washed in cold buffer, postfixed with 1% OsO_4 for 1 h, dehydrated in ethanol, embedded in Epon 812 and cut in an LKB Ultrotome III. One micron thick sections were stained with toluidine blue, and ultrathin sections were stained with uranyl acetate and lead citrate. Ultrathin sections on stainless steel grids were also stained with VIH followed by lead citrate according to Brissie et al. (1975) and Spicer et al. (1975).

Results

Case Reports

Case 1. A 74-year-old man with a previous history of a myocardial infarction in 1961 and a cystectomy because of a urinary bladder carcinoma in 1977, sought medical care in December 1978 because of a slow-growing mass in the back noticed for 3 months. The tumor, about the size of half a grapefruit, was located beneath the apex of the left scapula. On palpation it was firm, had an even surface and was slightly movable from the chest wall. A smaller similar lesion was noticed beneath the apex of the right scapula. Fine needle

aspiration examination of the left-sided tumor indicated a benign fibrous lesion. This lesion was surgically removed. No operation was performed on the right-sided tumor. At examination one year later there remained a small nodule at the site of the operated tumor, and the size of the right-sided mass was unchanged.

Case 2. A 70-year-old woman noticed in June 1980 a slow-growing mass in the back. By a family doctor the tumor was primarily interpreted as a lipoma. On admission at the orthopedic surgery department 6 months after the lesion was first noticed, a firm movable mass was found beneath the apex of the left scapula. Preoperatively an elastofibroma or a chronic bursitis was suspected. The tumor was extirpated with a margin of surrounding adipose tissue and muscles. There were no postoperative complications.

Case 3. A 66-year-old man had noticed for 2 years before admission in January 1981 a mass to the right in the back. At examination an ovoid tumor was palpated at the angulus of the right scapula. The tumor was movable from the chest wall. Primarily a lipoma was suspected, but the tumor was very firm on palpation, and radiograms revealed a dense lesion. The tumor was extirpated with a margin of surrounding muscle and adipose tissue. The post-operative course was uneventful.

Case 4. An 83-year-old man sought medical care in March 1981 for a slowly growing, painless mass in the back, noticed for at least a year. A firm tumor located beneath the apex of the right scapula was observed by the surgeon. The lesion was excised.

Pathology

Gross Appearance

The tumor masses of cases 1, 2, 3, and 4 measured $4 \times 3 \times 2$, $6 \times 3 \times 1.5$, $11 \times 6 \times 2.5$, and $4 \times 2.5 \times 2$ cm, respectively. They were all rather firm and revealed predominating grey-white fibrous areas and some adipose foci on the cut surface. No tumor capsule could be found.

Light Microscopic Appearance

Routine sections stained with hematoxylin and eosin revealed in all 4 cases dense connective tissue forming irregular broad sheets and infiltrating adipose tissue. The connective tissue was built up by bundles of fibers and sclerotic, hyaline masses of collagen and more intensely eosinophilic fibers and globules. These eosinophilic fibers and globules in the hematoxylin and eosin-stained sections lacked the double refraction seen in the collagen fibers in polarized light. They also stained yellow (picrinophilic) with the van Gieson method and were positively stained with all the elastin staining methods: Verhoeff's iron hematoxylin, Weigert's resorcin-fuchsin and Gomori's aldehyde-fuchsin (Fig. 1). In the 1 micron thick sections of Epon-embedded tissue this material

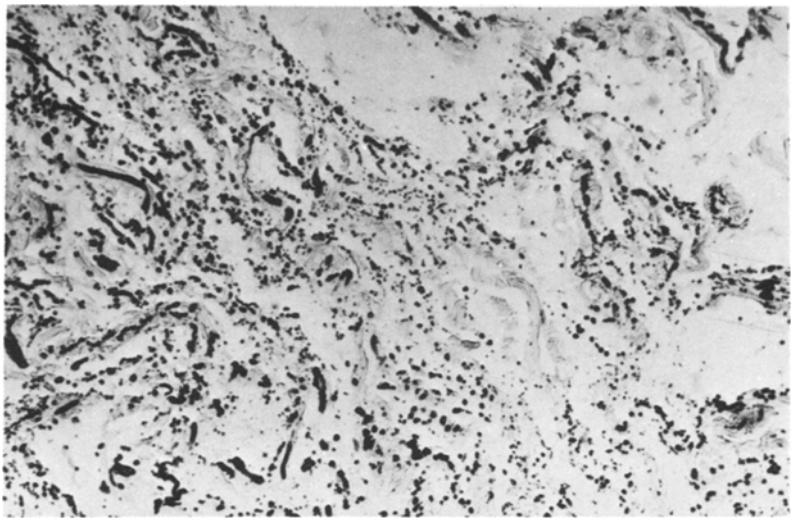

Fig. 1. Numerous intensely stained elastin bands and globular structures are seen mingling with collagen. VIH, ×90

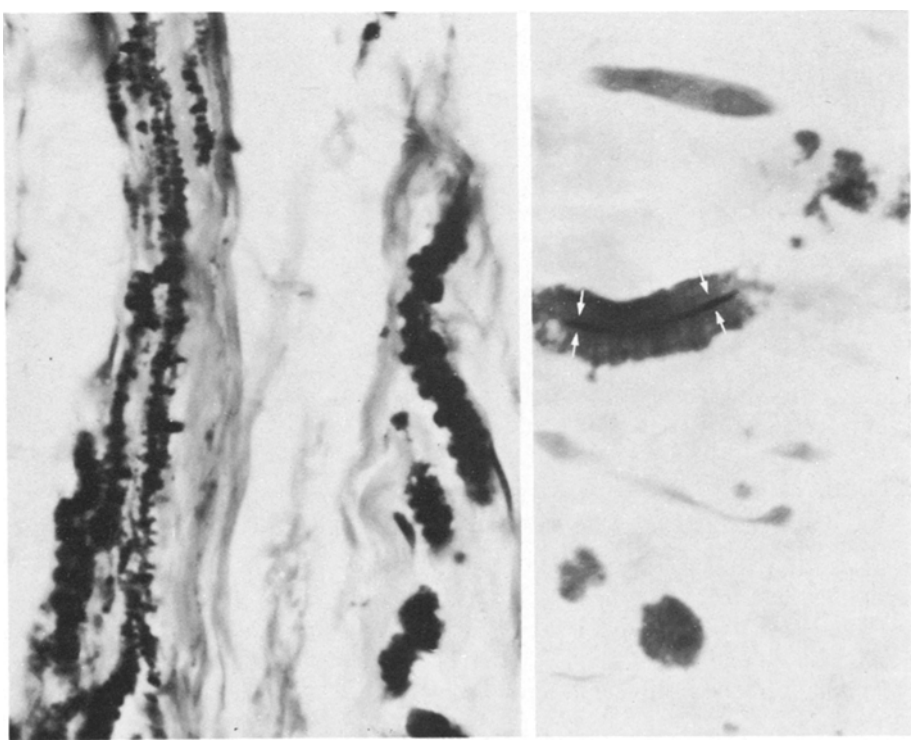

Fig. 2A, B. Elastin bands showing disc-like masses on an axis of dense material (*arrows*). One micron section. Toluidine blue, (**A**) ×350, (**B**) ×700

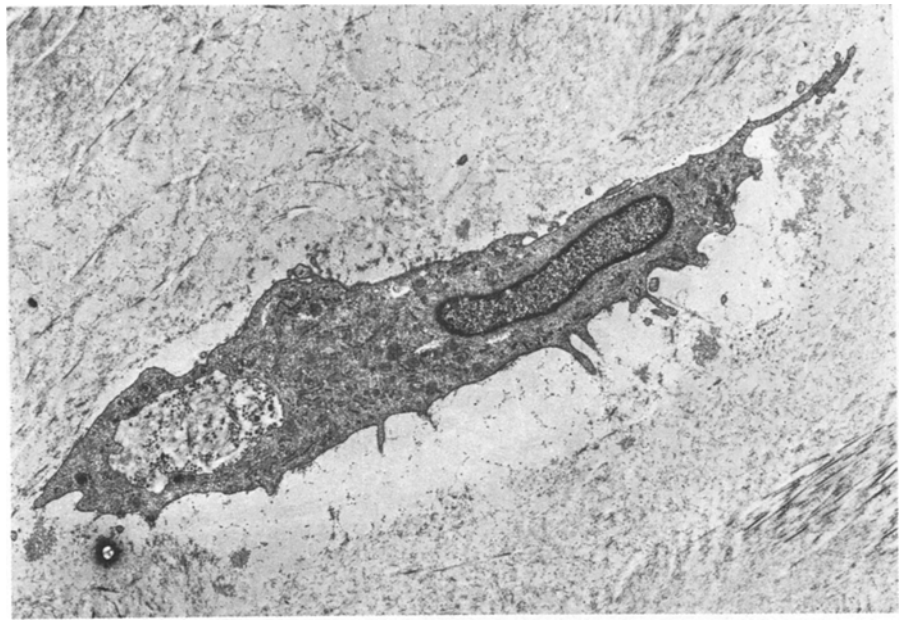

Fig. 3. Elongated fibroblast-like stromal cell with some cytoplasmic projections which is surrounded by and enclose bundles of collagen. ×7,500

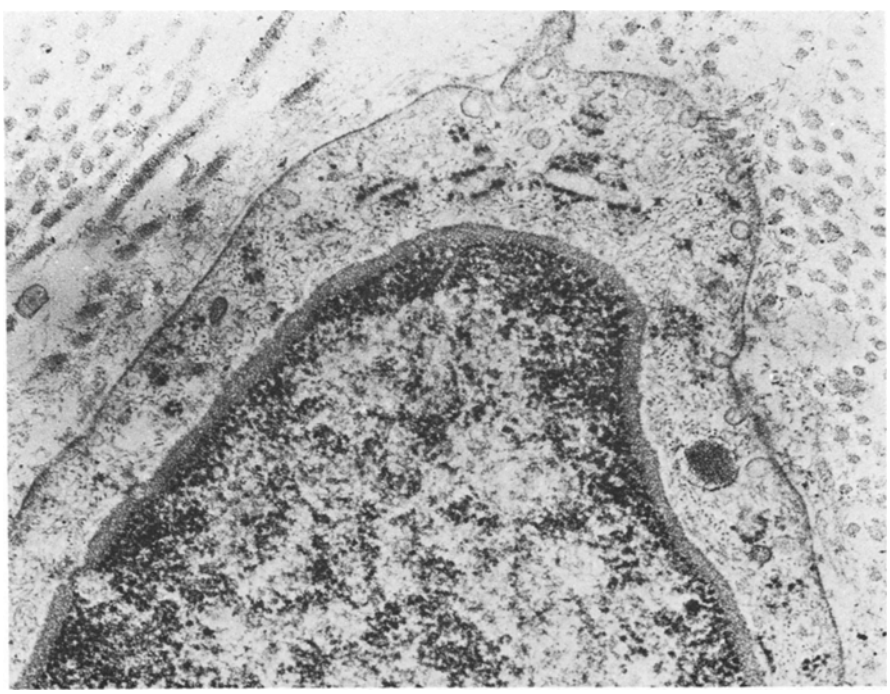

Fig. 4. Detail of a stromal cell showing prominent dense material in nuclear envelope. Several caveolae or pinocytic vesicles are seen at the plasma membrane. The plasma membrane is intimately associated with microfibrils and collagen. ×60,000

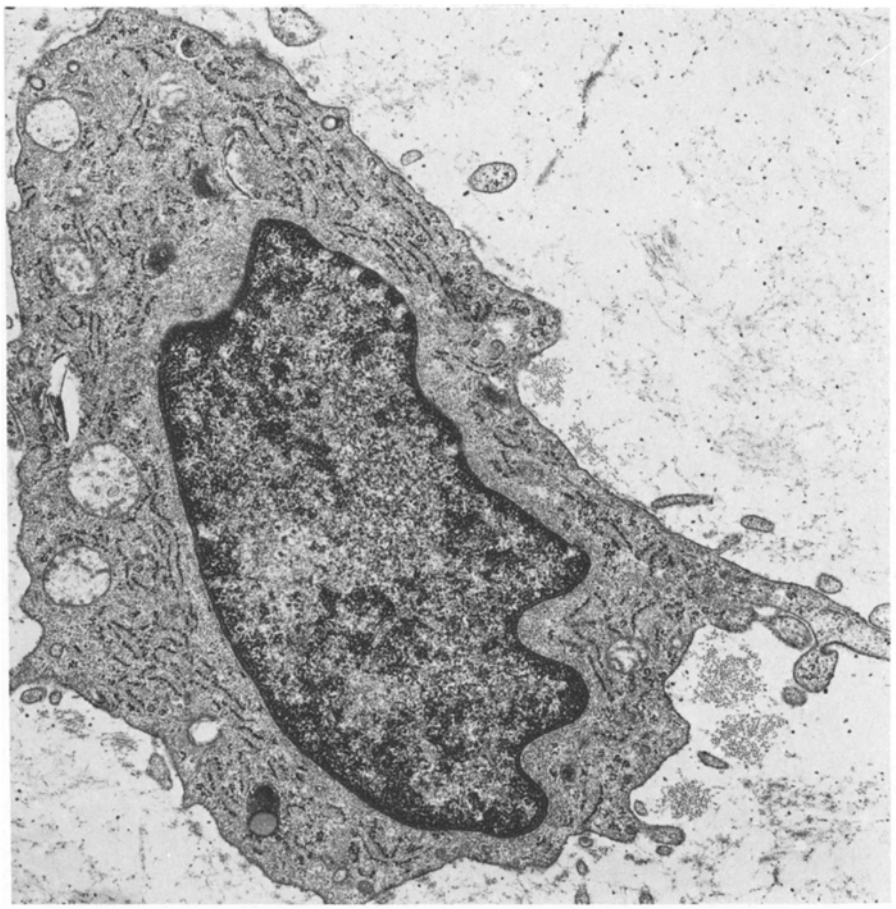

Fig. 5. Stromal cells showing perinuclear layer of cytoplasmic filaments and abundant RER. ×15,000

was intensely stained with toluidine blue; the structure and organization of the elastin material was most easily studied in these sections.

Most of the elastin material appeared in lower magnification as bands and globular structures of varying diameter. The broadest bands measured up to 30 microns in diameter. At high magnification the bands often revealed 1 or 2 central, intensely stained, homogeneous cords, 1–2 micron thick, along which mantles of elastin material were seen. These mantles were composed of periodic segments or discs giving the structures a necklace-like appearance (Fig. 2). The globules were composed of leaf-shaped structures which often surrounded a central cord of elastin staining material, giving these globules a flower-like appearance. Occasionally these central cords were fragmented and divided. Also many naked elastin fibers similar to the cords were seen; some of these naked fibers were continuous with the central cords within the elastin masses.

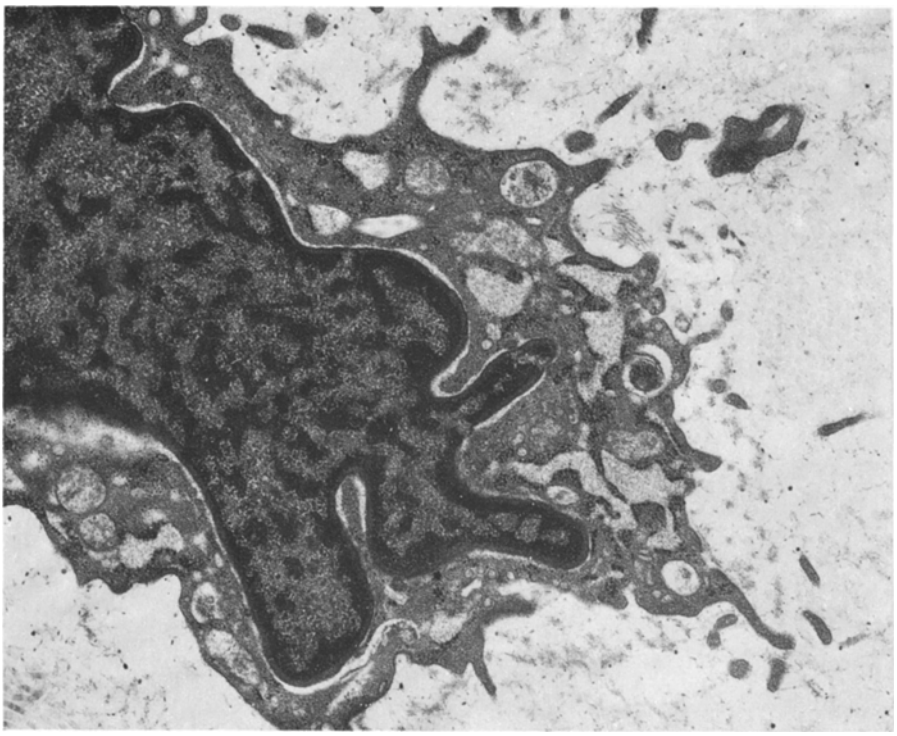

Fig. 6. Cell profile showing irregular cytoplasmic processes, wide cisternae of RER and a clefted nucleus with a prominent nuclear fibrous lamina. ×15,000

The arteries and veins within the lesion appeared mostly in the adipose tissue, but occasional larger mature vessels were also seen within the fibrous tissue. The veins in particular showed an increased amount of elastin, especially in the adventitia and perivascular tissues, which mingled with the abnormal elastin of the lesion.

Scattered uniform, spindle-shaped, fibroblast-like cells with elongated nuclei and slender cytoplasmic extensions were seen mingling with the collagen and elastin tissue fibers.

Electron Microscopic Appearance

The cell profiles scattered within the collagen and elastin stroma were fusiform, with often very prominent, uni- or bipolar cytoplasmic extensions (Fig. 3). The nuclei were elongated and often deeply clefted and showed a rim of condensed heterochromatin peripherally as well as clumps of internal chromatin and 1 or 2 prominent nucleoli. In many cells a layer of moderately dense material filled the nuclear envelope and enclosed the whole nucleus except for the nuclear pores (Fig. 4). The cytoplasm revealed parallel collapsed cisternae of rough endoplasmic reticulum (RER) and occasional widely dilated cisternae filled with

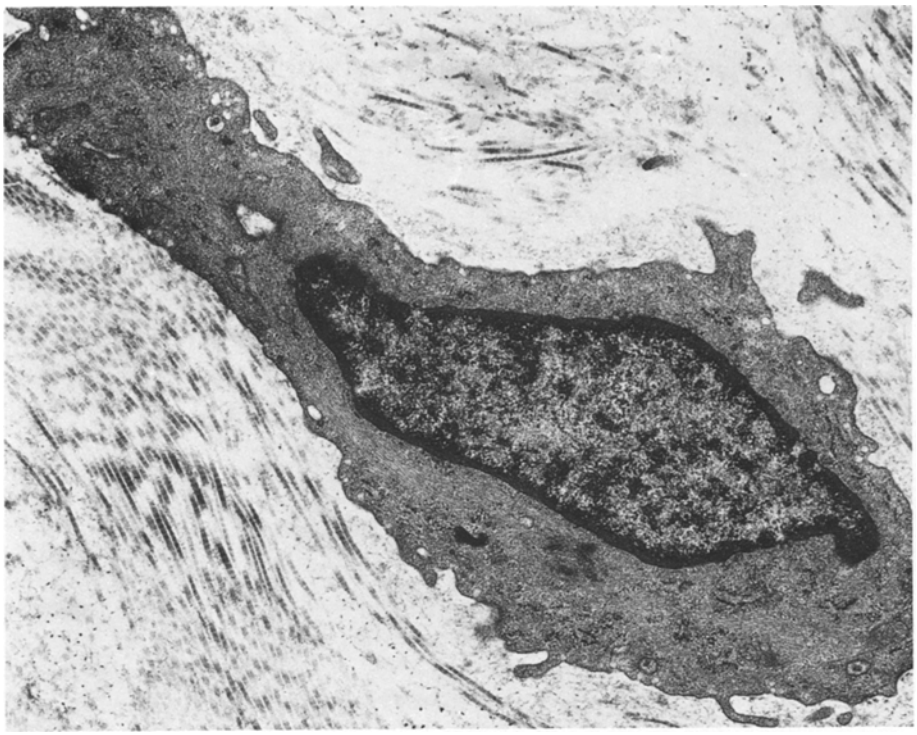

Fig. 7. Stromal cell with the cytoplasm almost completely filled with filaments arranged in parallel. ×15,000

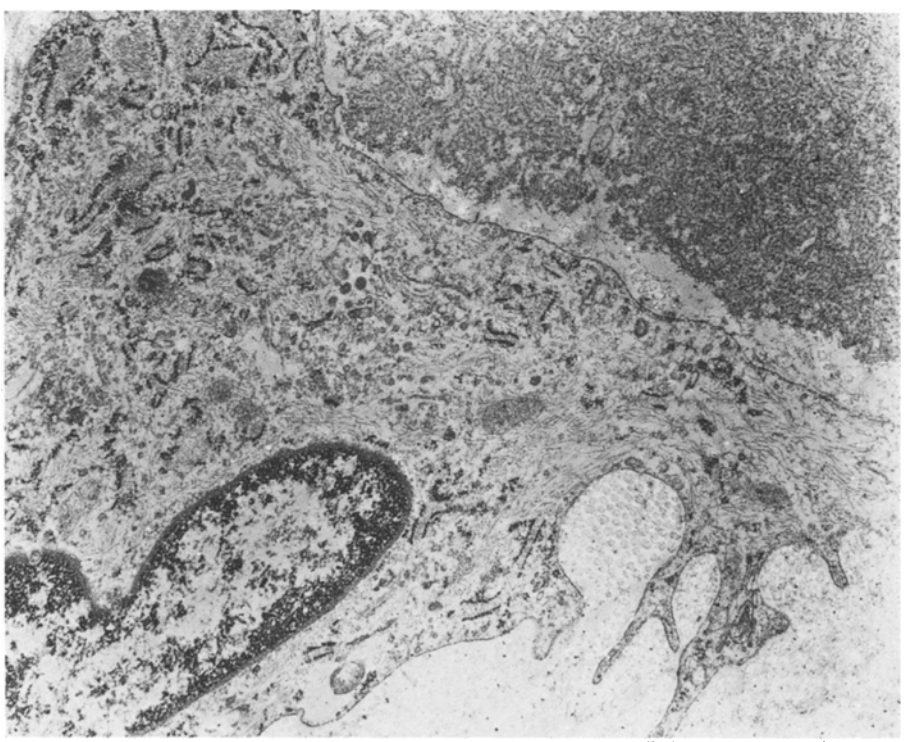

Fig. 8. Stromal cell associated with a large mass of elastin material (*top right*), microfilaments and collagen bundles. The elastin material shows a granular texture and stacked or whirled fibrils. ×25,000

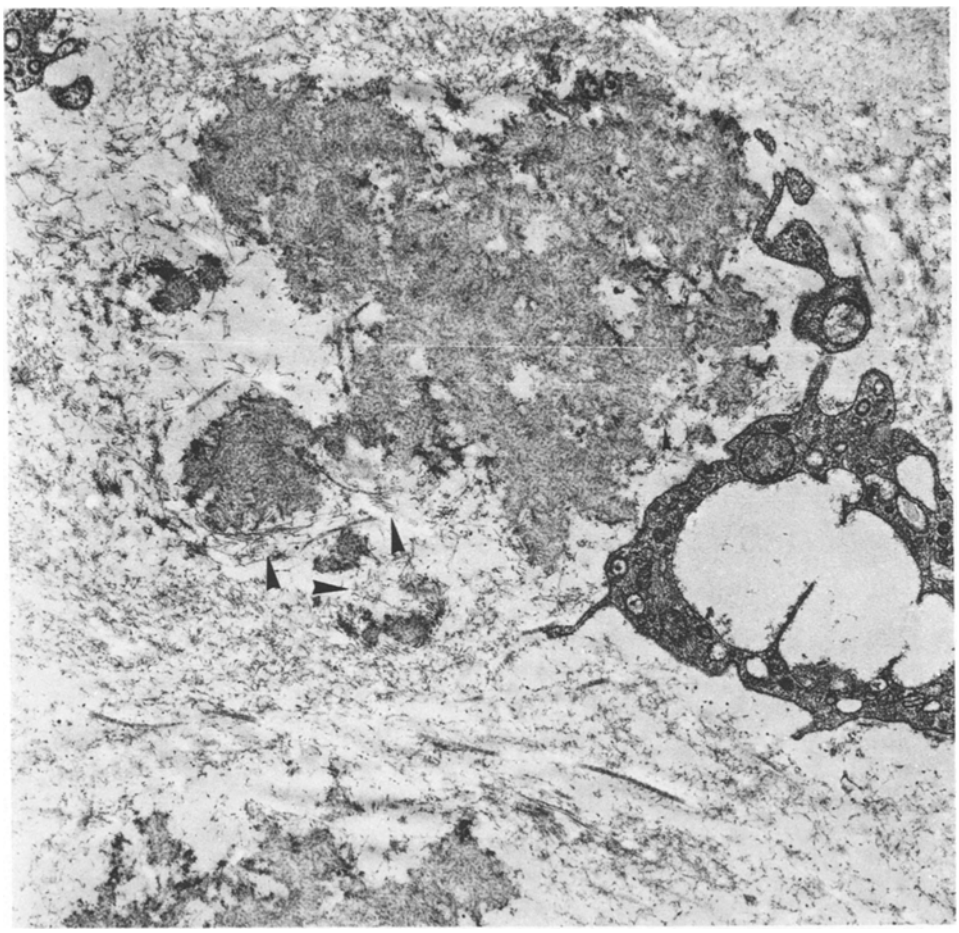

Fig. 9. A large loosely organized elastin mass bordered by cell processes. The elastin deposit shows small lucent holes and is at the periphery partly surrounded by fine microfibrils (*arrow heads*). ×15,000

a grey granular material together with scattered polysomes and free ribosomes. Rather few mitochondria were intimately associated with the RER; mostly they had a rounded profile and a lucent matrix and delicate cristae (Figs. 5 and 6). Cytoplasmic microfilaments arranged in parallel were abundant and filled most of the cytoplasm in some cells (Fig. 7). These microfilaments showed no longitudinal condensations or attachment sites. Along the cytoplasmic border numerous small pinocytic vesicles were seen. A common feature of the cells was their very long delicate cytoplasmic processes, often extending into the stroma far from the cell body. These processes often appeared as clusters of isolated small cytoplasmic extensions in an acellular stromal area. The processes showed pinocytic vesicles and were intimately associated with elastin material. Occasionally the cytoplasmic processes contained abundant glycogen. The cells lacked a basal lamina.

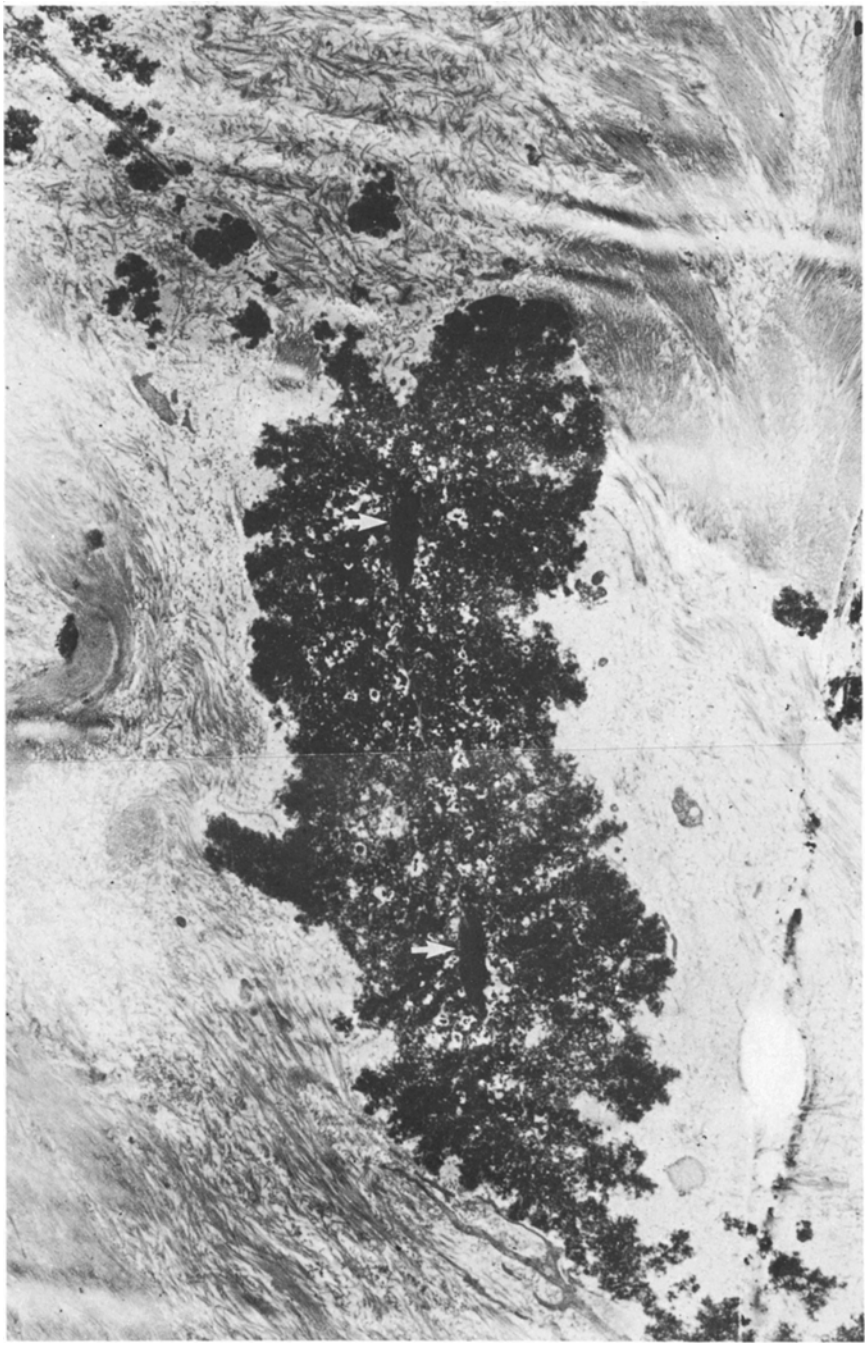

Fig. 10. VIH-lead citrate stained section with densely stained abnormal elastin material surrounding a central cord (*white arrows*). ×5,000

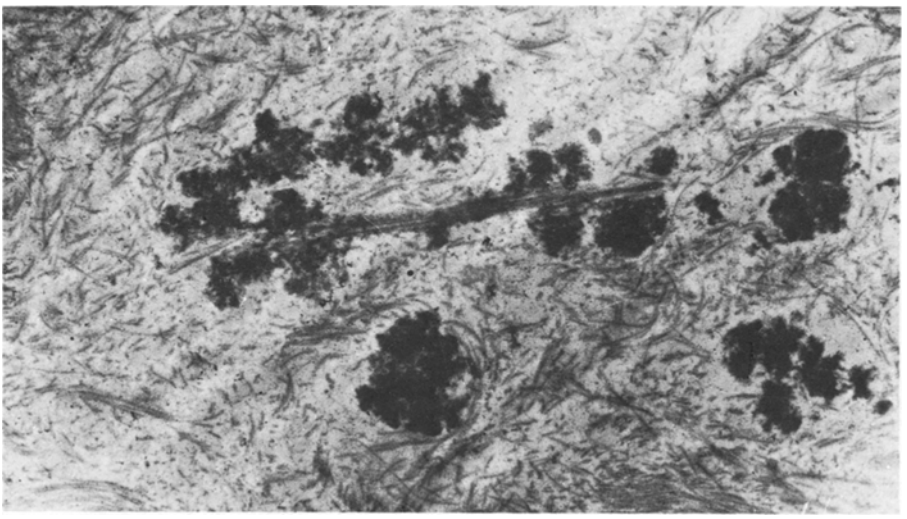

Fig. 11. Higher magnification of left upper corner of Fig. 10. A thin elastin fiber is partly surrounded by small globular masses of elastin material. ×15,000

The stroma was generally dominated by bundles of collagen fibers with ordinary periodic banding. The collagen mostly formed broad bundles of fibers arranged in parallel. In areas single, disorderly arranged collagen fibers predominated. Within the dense collagen stroma was abundant elastin material. In the routinely contrast stained sections this elastin appeared as irregular, abnormally dense masses of varying sizes, mingling irregularly with the collagen and often surrounded by weakly stained microfilaments (Figs. 8 and 9). At high magnification these areas appeared granular, revealing small granular structures closely packed or aligned in branching rows within a lucent background substance. Within these granular masses were small lucent holes, and the largest of these elastin masses often enclosed one large central lucent core.

In the VIH-stained sections the elastin material stained intensely, and small masses or fibers of elastin, not distinguishable in the routinely stained sections, could be identified. Also these sections showed the granular structure of the main part of the elastin material. Furthermore, the small lucent holes and the central lucent cores contained intensely stained material, which in longitudinal sections were disclosed to be strongly stained elastic fibers (Fig. 10). At the periphery of the elastin masses, more densely stained, delicate, branching filaments mingled with the surrounding collagen and the granular elastin material. Similar filaments were also observed within the granular elastin material. Rarely collagen fibers penetrated within the elastin material.

Numerous small and a few large, strongly stained, elastic fibers could be identified. These fibers were often completely surrounded by irregular small rounded masses of granular and fibrillar elastin material, but also fibers only partly or not at all surrounded by elastin material were seen (Fig. 11). There were also small globules of similar material irregularly mingling with the collagen and without any demonstrable relation to the elastin fibers.

Discussion

There are several reports in the literature dealing with the histogenesis of elastofibroma. Most studies have been based on light microscopic and/or histochemical examination, and occasionally electron microscopic investigation of a single case. The interpretations of these investigations are diverse and, although elastofibroma is morphologically and clinically well defined, the origin and the mechanisms of development are still disputed. It has been suggested that the broad fibers and globular structures of elastin staining material seen under the light microscope in fact represent elastotic degeneration of collagen (Stemmerman and Stout 1962; Barr 1966; Tighe et al. 1968). Alternatively the elastin structures could develop either as a degenerative change of preexisting elastin fibers or as a true elastodysplasia with new formation of elastic material (Winkelmann and Sams 1969; Akhtar and Miller 1977).

In the present study, the 4 elastofibromas revealed almost identical light and electron microscopic pictures. The VIH-staining of ultrathin sections made it possible to study the relationship between the various components of the elastic and connective tissue and helped to identify also very small deposits of elastin material, otherwise easily overlooked in the ordinary uranyl acetate and lead citrate stained sections. The combined light microscopic and electron microscopic examination of these tumors indicate that an abnormal, granular and fibrillar, elastin matrix material is actively formed in close relation to the spindle-shaped stroma cells. Although the lesion light microscopically is poor in cells, the ultrastructural investigation disclosed numerous delicate and very long cytoplasmic extensions, which were closely associated with the elastin material. Despite the absence of identifiable elastin material within these cells, this close relationship, the presence of cisternae of RER, filled with a grey, granular material, and the abundance of cytoplasmic membrane vesicles in the cell surfaces facing the elastin material, suggest the production and exocytosis of the essential precursors for elastin formation from these cells.

It has been suggested that the central cords seen by light and electron microscopy should be a sign of maturation of the new-formed elastin material into fibers (Akhtar and Miller 1977). The presence of naked fibers and fibers surrounded by granular elastin material only in small segments, however, rather speaks in favor of the opposite conclusion: that preexisting elastic fibers serve as a structural skeleton for the new formed elastin material (Järvi et al. 1969). The presence of numerous small elastin deposits without any relation to preexisting elastin fibers, however, indicates that such fibers are not necessary for the formation of the elastin matrix material but seem to be a requisite for the characteristic development of the necklace-like elastin bands.

Mostly at the periphery of the deposits of granular elastin material microfilaments and occasional enclosed collagen fibers were observed. This has been shown to be a common finding also in ordinary elastogenesis and should therefore not be considered as an evidence of elastotic degeneration of collagen (Haust and More 1967).

A characteristic feature of the nuclei of the spindle-shaped stroma cells was the presence of a band of fine-textured medium density material, adjacent

to the inner membrane of the nuclear envelope, so called nuclear fibrous lamina. It has been shown by Ghadially (1980) that the nuclear fibrous lamina is not a static structure for a given cell type, but that it is a dynamic component of the nucleus, capable of undergoing hypertrophy and involution in physiologically and pathologically altered states. A thickening of the nuclear fibrous lamina has been noted in repair tissue filling surgically produced defects in articular cartilage and in the synovial intimal cells in cases of rheumatoid arthritis. From these studies Ghadially and coworkers have concluded that the fibrous lamina may be an indicator of a repair reaction. Such fibrous laminae have been observed in myofibroblasts from repair tissue. In this connection, it is of interest to notice that the spindle-shaped stroma cells contained abundant cytoplasmic thin filaments of actin type arranged in parallel with the long axis of the cells, i.e. features similar to that seen in myofibroblasts. However, the filaments showed no focal densities or attachment sites and no basement membrane material was seen as in most myofibroblasts. Similar cells have been described in an elastofibroma by Ramos and coworkers (1978). Myofibroblasts have been identified in a large variety of lesions, predominantly reactive processes and benign or low grade malignancy neoplasms (Seemayer et al. 1980; Bhawan 1981). The presence of prominent nuclear fibrous laminae and the partly myofibroblast-like appearance of the stromal cells may therefore further support the opinion that elastofibroma should be considered as a reactive, non-neoplastic lesion, characterized by active synthesis of elastin material, predominantly laid down around preexisting elastic fibers.

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