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Electron Microscopic Findings in Four Cases of Nasopharyngeal Fibroma

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Summary. Light and electron microscopic investigations of four cases of juvenile nasopharyngeal fibroma revealed characteristic structures; a fibrous stroma, an inclination to hyalinisation and formation of scar like tissue, a lacunar thin walled vascular component, large numbers of mast cells and of fibroblasts. The tumor fibroblasts contained different nuclear bodies and particles. There existed five different types of more or less complex spherical bodies. The previously described nuclear electron dense particles with an electron lucent halo could be divided into four groups measuring 60, 90, 150 and 300 nm in diameter.

In addition to the previously described ultrastructural properties of the tumors, the nuclei of the tumor fibroblasts were found to contain virus like particles. These particles were less electron dense, measuring 40 to 55 nm in diameter and arranged in groups throughout the nucleoplasm; they were different from chromatin condensations and from perichromatin granules. The structure and the size of the smallest particles was not comparable with the other nuclear inclusions.

Key words: Nasopharyngeal fibroma – Nuclear inclusions – Virus-like particles.

Introduction

Nasopharyngeal fibromas (juvenile angiofibromas) are regarded as histo-cytologically benign, locally invasive soft tissue tumors affecting mainly adolescent males. A higher incidence of these tumors is well established for some regions of Mexico, Egypt, India, Southeast Asia, and Kenya (Gatumbi and Linsell, 1964; Acuna, 1973). The clinical course and pathological features of the neoplasm have been reviewed by several authors collecting up to 120 cases (Shaheen, 1930; Friedberg, 1940; Figi and Davis, 1950; Sternberg, 1954; Erich, 1955;

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Schiff, 1959; Härmä, 1959; Apostol and Frazell, 1965; Arold and Schätzle, 1971; Neel et al., 1973).
Electron microscopic descriptions of nasal juvenile fibromas have been presented by Svoboda and Kirchner (1966), McGavran et al. (1969), Walike and Mackay (1970), Dorn et al. (1971), Seifert (1971), Küttner (1972/73), Nowak et al. (1974), Stiller et al. (1976), and Taxy (1977). Ultrastructural examination revealed intranuclear granules of different size and varying electron density in the stromal cells of the tumor. These observations have lead to speculation about the specifity of the inclusions and their resemblance to virus particles.

The finding of previously undescribed particles within the nuclei of tumorous fibroblasts together with nuclear granules of the type previously described and the observation of specific cytological findings in nasopharyngeal tumors provided the rationale for intensive electron microscopic examinations in four own cases.

Material and Methods

Morphological investigations were carried out on specimens of four nasopharyngeal fibromas from males between eleven and sixteen years of age.

Light microscopy was performed using semithin sections treated with paraphenyldiamine and Alcian–PAS and in sections of formalin fixed tissue embedded in paraffin, stained with Giemsa, hematoxylin-eosin, Iron hematoxylin-picrofuchsin Van Gieson with resorcin, Goldner's modification of Masson's trichrome, Alcian-PAS reaction, and the Feulgen reaction.

For electronmicroscopy specimens were fixed in 3,4% buffered glutaraldehyde with postfixation in 1% osmium tetroxide. Before embedding in Epon the pieces were treated with uranyl acetate. The ultrathin sections were partly investigated following treatment with lead citrate.

Light Microscopic Findings

In the *first* case the mucous epithelium covered superfically oedematous connective tissue. No limiting membrane nor capsular tissue could be detected between the superficial stroma and the adjacent tumorous tissue. The subepithelial connective tissue was infiltrated by polymorphonuclear leukocytes, lymphocytes, and histiocytes while the tumor itself included only small groups of inflammatory cells. The tumor consisted of fine and coarse collagen fibrous material with wavy fibrous bands, scar like area and numerous vessels of capillary (Fig. 1a, b) venous and arteriolar type. The fibroblastic cells between the fibers were characterized by an angular or stellate cytoplasm and plump occasionally invaginated nuclei (Fig. 1c). The nuclei often contained two or three nuclear bodies and a bubbly chromatin structure. Following the Feulgen reaction nucleoli disappeared and the other chromatin assumed a rough vacuolar appearance. The tumor center contained numerous mast cells (Fig. 1d). Scattered metachromatic granules were present lying free between the collagen fibers and adjacent vascular walls. Next to the granules the fibrous network appeared loosened.

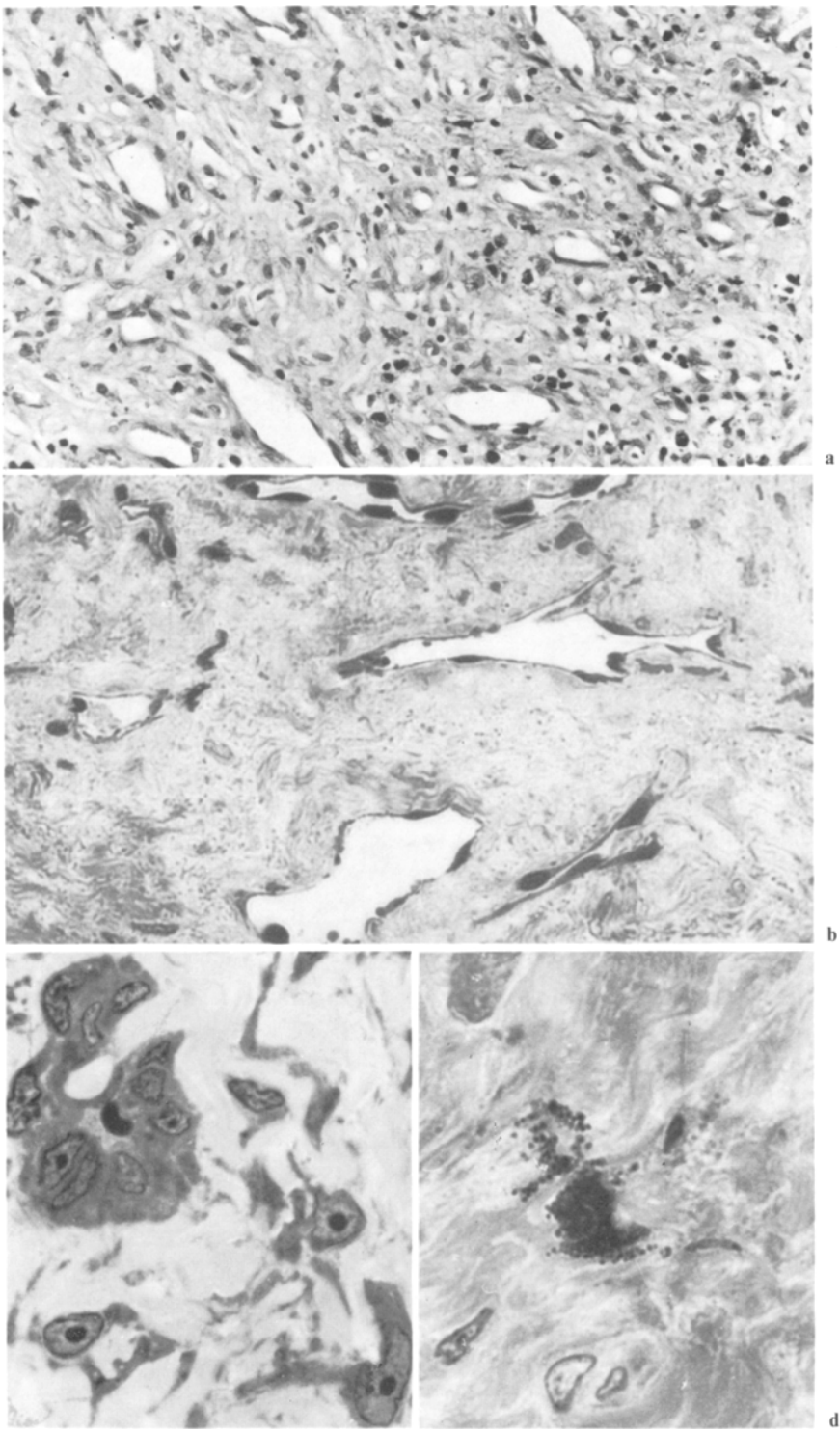


Fig. 1. a Light microscopic survey of a nasopharyngeal fibroma with fibrous stroma and numerous vessels. H & E, ×300. **b** Phase contrast micrograph of a semithin section with lacunar thin walled vessels and surrounding scar like connective tissue. ×480. **c** Tangential section of a blood capillary with sprouting angioblasts surrounded by collagen and fibroblasts with large nuclei and prominent nucleoli. Semithin section, Mallory, ×1200. **d** Phase contrast micrograph of fibrous tumor stroma with a mast cell containing numerous granules. Free mast cell granules in the adjacent stroma. ×1200

The *second* and *third* cases resembled the tissue described above, being surrounded by granulomatous tissue of a non-specific type and of mucous epithelium. There was a more marked development of scar like tumor tissue within the central parts in comparison with the first case (Fig. 1b). The vascular walls showed an increase in medial smooth muscle cells. The center of this tumor also exhibited mast cells with dense granulation.

The *fourth* case had a similar histological structure, apart from an inflammatory reaction in the outer layers. The neoplastic tissue had many large nuclei with granular or coarse vacuolar structures. The vessels of this tumor were partly lacunar, sometimes surrounding small aggregates of platelets, with continuation into angioblastic sprouts. Each tumor specimen showed a disparity between the degree of vascularisation and the amount of blood present; some parts appeared almost anemic. In all cases the tumors were devoid of capsule and surrounding basal lamina.

Electron Microscopic Findings

Electron microscopically the tumor cells were surrounded by dense bundles of collagen fibers with regular periodicity (Fig. 2, 3a, 4). Occasionally smaller elastic fibrillary material and smaller fibers adjacent to the cells and to mature collagen fibers could be detected. In some areas the smaller fibers were connected to cells by numerous filaments (Fig. 3a, 6). Bending of filamentary material from the cells into the surrounding collagen war observed. Within some fibroblasts invaginations of mature collagen fibers were visible (Fig. 4). The tumorous fibroblasts contained ergastoplasmic tubules and free ribosomes (Fig. 4, 5). Ergastoplasmic cisternae were sometimes dilated and filled up with a fine granular substance (Fig. 5). An intensive microvesiculation was prominent along the cellular membrane (Fig. 4). Mitochondria of ovoid shape and of cristae type and lysosomes, were all of medium size and normal appearance. Some cells included small lipid droplets without a limiting membrane. Frequently, a Golgi complex was well developed including granular rosettes. Mitotic figures were visible in different stages. Case two and three showed less fibroblastic elements these being partly replaced by slim fibrocytes with a less well defined cytoplasmic structure. Regressive changes in fibrocytes were seen. Loosened parts of the fibrous tissue were intermingled with microfibrillar structures and free cellular organelles. The nuclei of the tumor fibroblasts were pleomorphic and often invaginated (Fig. 5, 6). The nuclear cisterna was widened and its ribosomal layer continued into the cytoplasmic ground substance. The ribosomal layer seemed to be condensed within the cells, including the increased filamentary structures (Fig. 6).

The nuclei of the tumor fibroblasts contained particles of varying diameter and of high electron density. They were surrounded with a pale halo measuring 11 nm in diameter (Fig. 6, 8). Their matrix consisted of a uniform fine granular material, and they were not equipped with any capsular structures or core-like condensations. These intranuclear particles, which were distributed throughout the total nucleoplasm could be roughly divided into four groups:

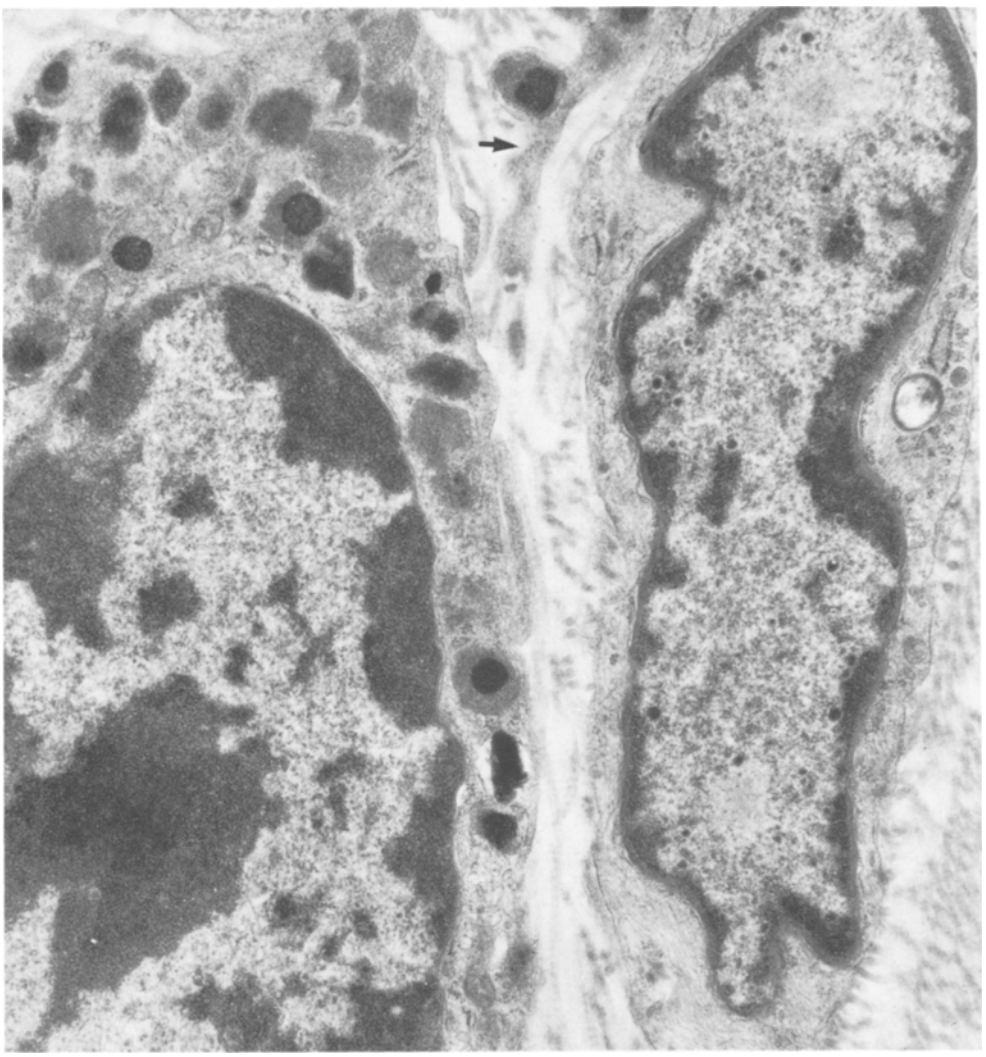


Fig. 2. Close contact of a fibroblast and a mast cell with mature granules and a fingerlike cytoplasmic protrusion (→). The fibroblast nucleus contains dense particles of different size. ×23,000

Group one represented particles with an average diameter of 60 nm (Fig. 2, 5), and the second group included particles with an average diameter of 90 nm (Figs. 2, 4–6). Particles of these two groups were the most frequent inclusions. The diameter of the third type ranged between 120 and 150 nm (Figs. 4, 6, 8). A fourth group of giant electron dense particles of identical composition measuring up to 300 nm in diameter (Fig. 8) occurred within nuclei of degenerating fibrocytes.

Another type of intranuclear particle also occurred exclusively within tumor fibroblasts, being found in each third or fifth nucleus in all four cases. Their diameter ranged between 40 and 55 nm. These small particles were less electron

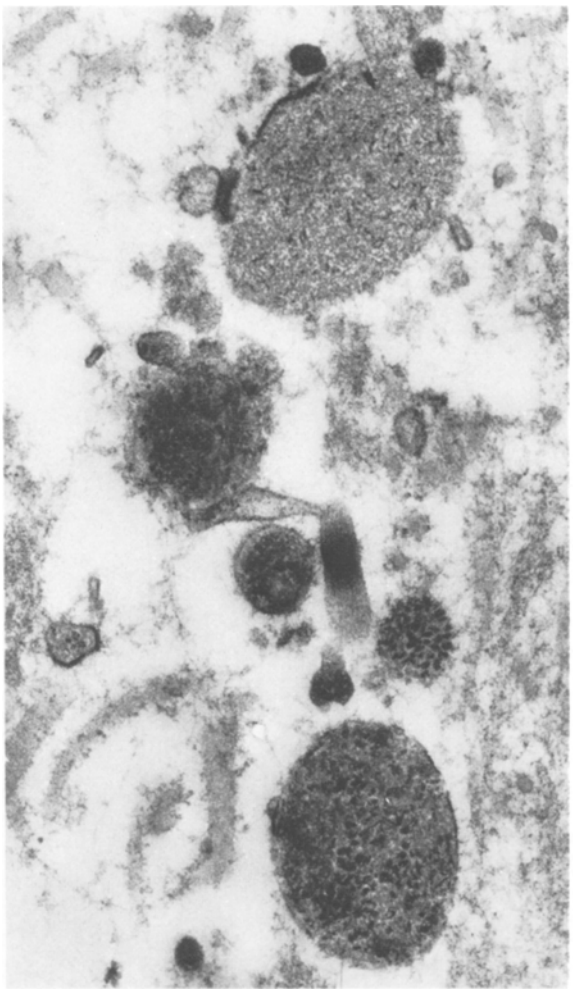
a

Fig. 3.a Degenerating mast cell next to loosening collagen tissue and fibrolysis. ×23,000. **b** Free mast cell granules between loosened collagen fibers. ×62,000

dense and also of granular structure. They always appeared in aggregates of fifteen to twenty five particles within one cross section (Fig. 8). These smallest particles did not appear after omission of lead citrate staining. The aggregates were irregularly distributed among the nuclear chromatin without any particular relationship to the perichromatin or to the nucleoli. No apparent release of particles from the nuclei into the cytoplasm was observed.

The nuclei of the tumor cells also contained spherical bodies measuring from 380 to 520 nm in diameter (Figs. 6, 7). These were composed of fine fibrillar material, sometimes with a whorled appearance. Electron density varied but the bodies were usually surrounded by a lucent halo.

The mast cells contained numerous round and oval cytoplasmic granules with a homogeneous, lamellar, or filamentary interior and paracrystaline inclusions (Figs. 2, 3a). Similar granules could be demonstrated between the collagen-



b

eous stroma of the tumors (Fig. 3b). Next to the free mast cell granules some poorly defined cytoplasmic processes of mast cells were evident (Fig. 3a, b). Between free granules the collagen fiber network seemed to be loosened up. The nuclei of the mast cells were free of the inclusions described above. Some of the mast cells were in close contact with fibroblasts where degeneration of fibroblasts resp. of fibrocytes was observed with protrusions of the cellular membrane and dissolution of their membrane.

Smooth muscle cells were only seen associated with the vascular tumor components. Occasionally cells with myofibrils between tumor fibrillary stroma were found to be of vascular origin after examination of serial sections.

Lymph vessels did not occur within the tumors. Capillaries were formed of prominent endothelial cells with large nuclei, often continuing into angioblastic elements, and some of the endothelial cells contained bundles of fine fila-

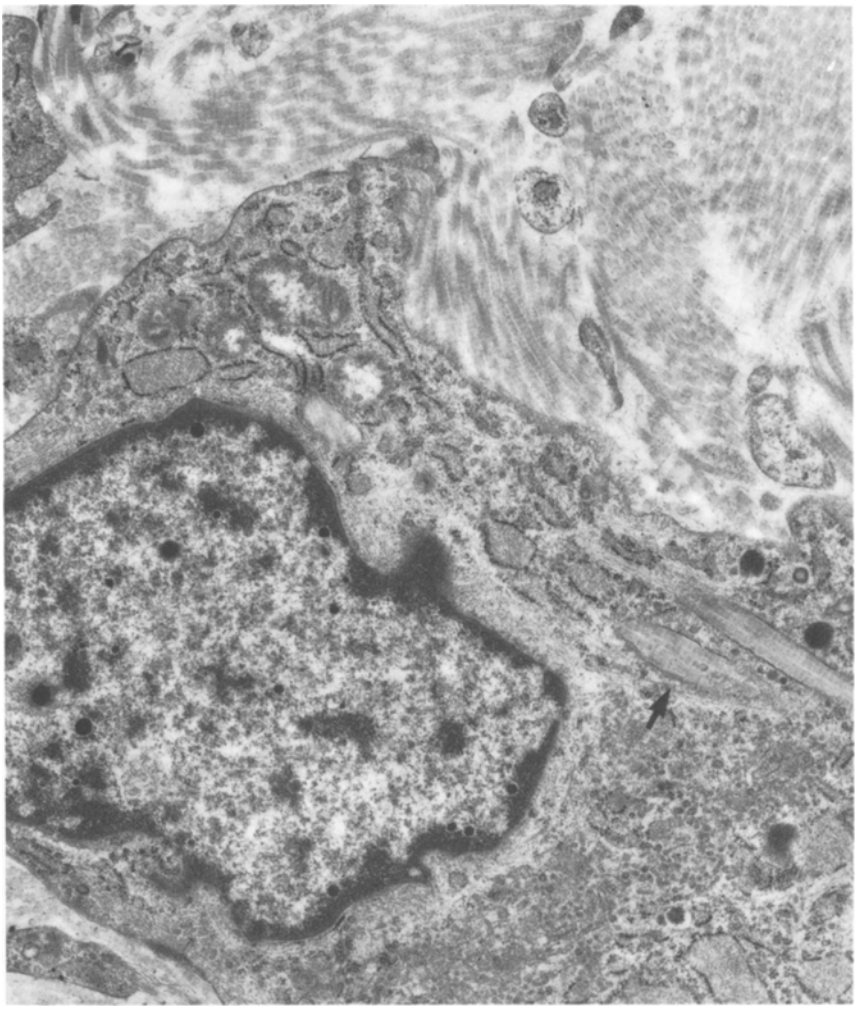


Fig. 4. Typical fibroblast with electron dense nuclear particles and cytoplasmic cross sections of invaginated collagen fibers. (*arrow*) ×15,000

ments. A continous basal lamina surrounded the endothelial cell layer. Endothelial cells and vascular smooth muscle cells of the larger blood vessels were free of intranuclear inclusions.

Discussion

The light microscopic findings of the presented cases resemble earlier extensive descriptions of nasopharyngeal fibromas given by Sternberg (1954), Handousa et al. (1958), Osborn (1959), Härmä (1959), and Schiff (1959). Regressive changes in the tumor with development of scar like tissue, hyalinized foci and sponta-

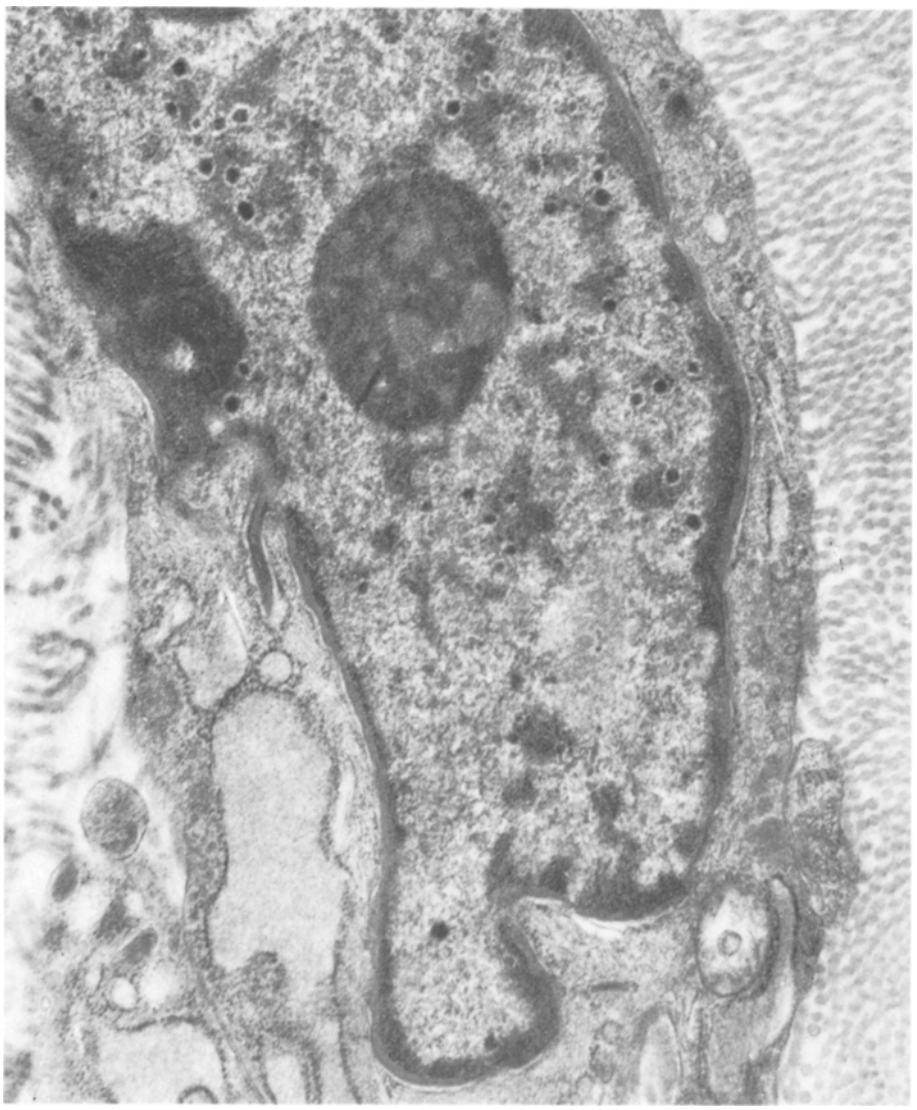


Fig. 5. Tumor fibroblast with dilated ergastoplasm and numerous nuclear particles with high electron density. ×23,000

neous remission are well documented. The small amount of blood within the lacunar tumor vessels in comparison with the total vascular diameter might be due to a sphincter arrangement with vascular constriction as discussed by Svoboda and Kirchner (1966). The presence of fibroblastic cells and large numbers of mast cells can be regarded as one of the main characteristics of nasopharyngeal fibromas. The mast cells are of the type with mature granules, the release of which seems to influence the coherence of the surrounding connec-

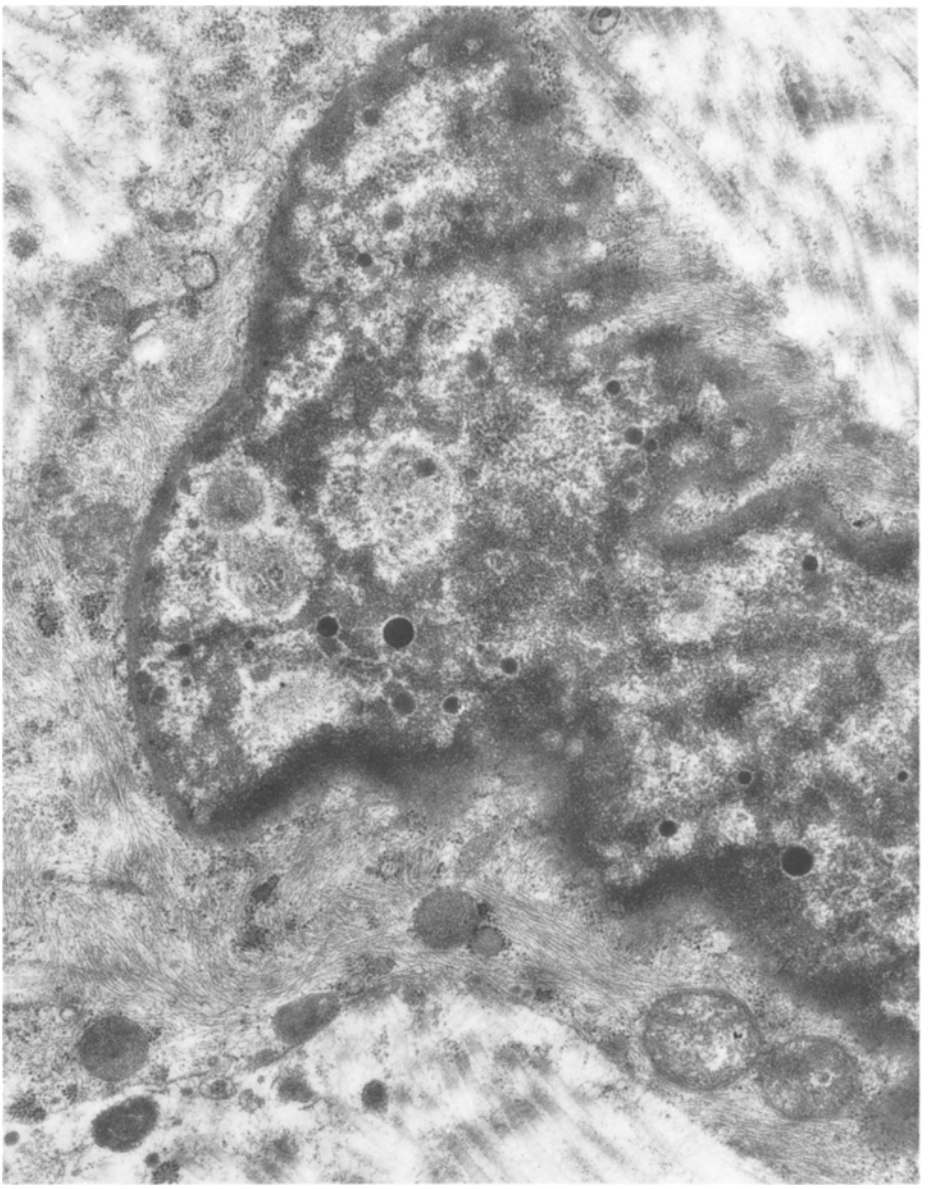


Fig. 6. Degenerating fibroblast with several spherical nuclear bodies and electron dense nuclear particles of different diameter. The cytoplasm with dense filamentary structure. ×23,000

tive tissue; degeneration of fibrocytes and splitting of elementary fibrils to protofibrils as described by Gieseking (1966) is apparent.

An increase and condensation of intracytoplasmic filamentary material together with a condensation of ribosomes at the outer nuclear membrane, with final dissolution of this membrane and release of cellular organelles to the

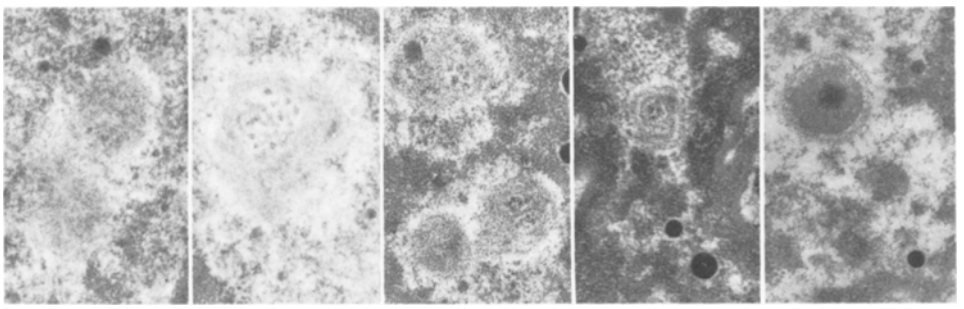


Fig. 7. Different types of spherical nuclear bodies. ×23,000

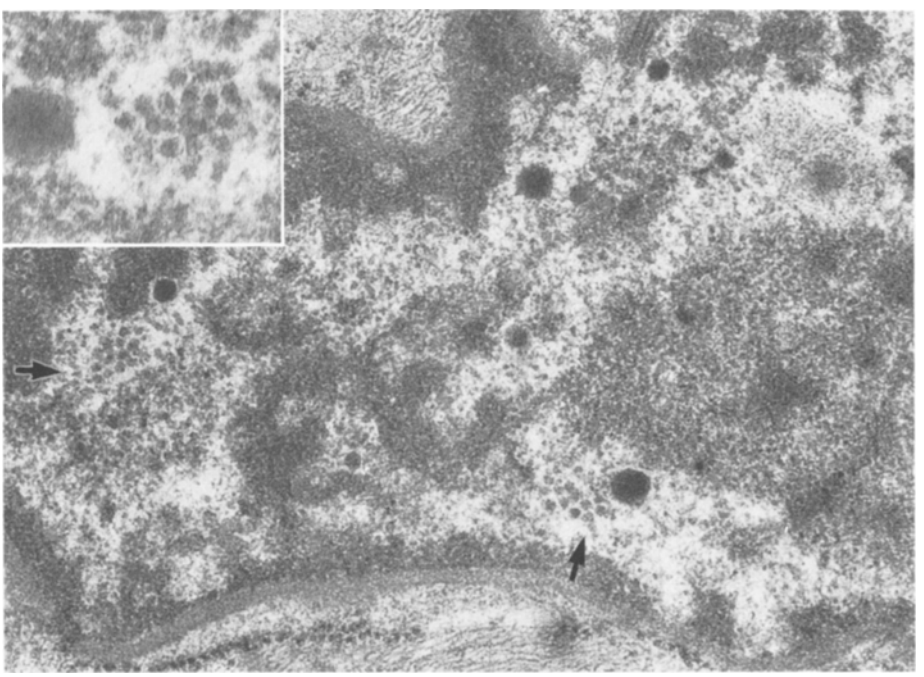


Fig. 8. Aggregates of small virus like nuclear particles (→) next to larger electron dense inclusions. ×43,000. Inset: Higher magnification of an aggregate of small particles. ×85,000

extracellular tissue, is regarded as a further process of degeneration and aging within the tumor.

The vascular component of the fibroma consists of lacunar capillaries and of small arterioles and venules. In contrast to findings of Taxy (1977) cells with myofibrils were only seen in connection with vessel walls. Analogous to the observations of Seifert (1971) an angioblastic proliferation was demonstrated next to several tumor capillaries, and intensive fibrillogenesis was only related to the tumor fibroblasts.

Sex and age linked occurrence and the finding of a higher incidence of gonadal insufficiency among patients with nasopharyngeal fibroma all underline the importance of endogenous factors for the growth of the tumor. However the geographical relationship of the occurrence of the tumor with almost endemic occurence in some regions suggest a contact possibly viral pathogens. In addition, other tumors with age linked manifestation (laryngeal papillomas or dermal verrucae) contain virus particles (Dmochowski et al., 1964; Nasemann, 1974; Arnold, 1976; Arnold et al., 1977). From this point of view the demonstration of intranuclear inclusions similar to virus particles is of interest.

Intranuclear spherical bodies with variable appearances and occasionally complex structure were found in numerous fibroblasts of the tumors and corresponded with the five groups described by Bouteille et al. (1967). Identical nuclear bodies have been demonstrated in degenerating squamous cells of human laryngeal papillomas (Arnold, 1976; Arnold et al., 1977). Horstmann and coworkers (1966) considered similar bodies to be non-pathologically significant findings but Bouteille and coworkers point out that these structures appear frequently in tumors and viral diseases. According to Robertson and McLean (1965) and Henry and Petts (1969) spherical nuclear bodies are generally seen in rapidly growing tissues with a high rate of cellular division. Krishan et al. (1967) considered the bodies to be RNA- and DNA-free protein and histone containing structures, and agreed that they are seen in many tumors, in cells with virus infection and following irradiation.

Intranuclear particles with an electron dense fine granular matrix and a lucent halo have been described as pathognomonic for nasopharyngeal fibromas by Svoboda and Kirchner (1966), McGavran et al. (1969), Albrecht et al. (1970), Walike and Mackay (1970), Seifert (1971), Dorn et al. (1971), Stiller et al. (1976) and by Taxy (1977). In contrast to Nowak et al. (1974) we did not see these particles within the cytoplasm. As described the particles were divided into four groups. The "giant" electron dense particles, measuring up to 300 nm in diameter, were only seen in degenerating fibroblasts. The particles were never seen within nuclei of the tumor mast cells or those of the vascular wall constituents.

The ultra-histochemical studies of Küttner (1972/73) have suggested the particles are albumen free of DNA and RNA. We can not add further information concerning their composition.

We have also demonstrated a hitherto undescribed type of particle in the nuclei of the tumor fibroblasts. These particles measure between 40 and 55 nm in diameter and are arranged in groups of 15 to 25 particles within one cross section. These smallest particles were less electron dense in comparison to the particles with an electron lucent halo discussed above.

The diameter and fine structure of the smallest particles were comparable to those of papova-viruses (Howatson, 1973). In contrast to perichromatin granules, these particles have a constant size and a more sharply defined outline; in addition, they were spread over the total nucleoplasm. The particles could be considered to be condensations of hypertrophied ribonucleoprotein granules (Haguenau, 1973) but their shape and arrangement suggest a more virus like nature. It should be pointed out that the smallest particles were seen in at least every fifth cell, being found more often in degenerating tumor cells. The

hypothesis of a virus infection of tumor fibroblasts is supported by the intranuclear grouped appearance and the increase of bodies with spherical shape in the nuclei. In addition, clinical observations of almost endemic manifestations of the nasopharyngeal fibroma in some geographical regions provides circumstantial support.

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