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Cellular Differentiations and Structural Characteristics in Nasopharyngeal Angiofibromas

An Electron-Microscopic Study

Dankwart Stiller, Detlef Katenkamp, and Klaus Küttner

Institute of Pathology (Head: Prof. Dr. F. Bolck) and Clinic of Oto-Rhino-Laryngology (Head: Prof. Dr. R. Albrecht) of the Friedrich-Schiller-University of Jena/DDR

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Summary. An electron-microscopic study of 9 nasopharyngeal angiofibromas was performed in order to elucidate the ultrastructural characteristics. Stromal fibroblasts and proliferating cells of the microvasculature were found. The stromal fibroblasts were subdivided into 3 different groups: (1) "classical" fibroblasts, (2) fibroblasts with histiocytelike features, and (3) fibroblasts with myoid features. By proliferation the cells of the capillary vessels change into stromal cells. A particular pattern of nuclei and dense intranuclear granules is only found in stromal fibroblasts. Consequently fibroblasts as well as cells of the microvasculature contribute to the pool of tumor cells.

Key words: Nasopharyngeal angiofibroma — Fibroblast — Vascular cells — Fibromatosis — Electron microscopy.

Nasopharyngeal angiofibromas (basal fibroid, juvenile angiofibroma) reveal typical histologic pictures, but show certain structural variegations. With regard to their classification into the fibromatoses (Enzinger et al., 1969), detailed ultrastructural analyses are necessary. Electron-microscopic examinations presented in the literature preferably described the intranuclear dense granules (Svoboda and Kirchner, 1966; Albrecht and Küttner, 1970; Dorn et al., 1971; Seifert, 1971).

Material and Methods

Forty nasopharyngeal angiofibromas supplied by the collection of Prof. Zange and Prof. Albrecht were studied by light microscopy. Tissue samples of 9 nasopharyngeal angiofibromas were examined electron microscopically. The patients were 7–24 years old. Fixation of specimen samples in a mixture of 1.25% glutaraldehyde and 1.5% formaldehyde buffered by 0.1 M phosphate buffer at pH 7.4. Postfixation with 2% OsO_4. Further preparation in the usual manner.

Results

Light microscopy shows different densities of cells and vessels even within the same lesions (Fig. 1). Generally the lesions resemble erectile tissues or cavernous hemangiomas with abundant fibrous stroma. The stromal cells are similar to stellate fibroblasts. The nuclei of the cells are of a plump shape and the multiple cytoplasmic processes are faintly stainable. There are sometimes edematous places in the fibrous tissue. In some areas collagen fibers are abundant and arranged in coarse irregularly interlacing bundles or parallel wavy bands. Furthermore, discrete hyalinized foci can be seen.

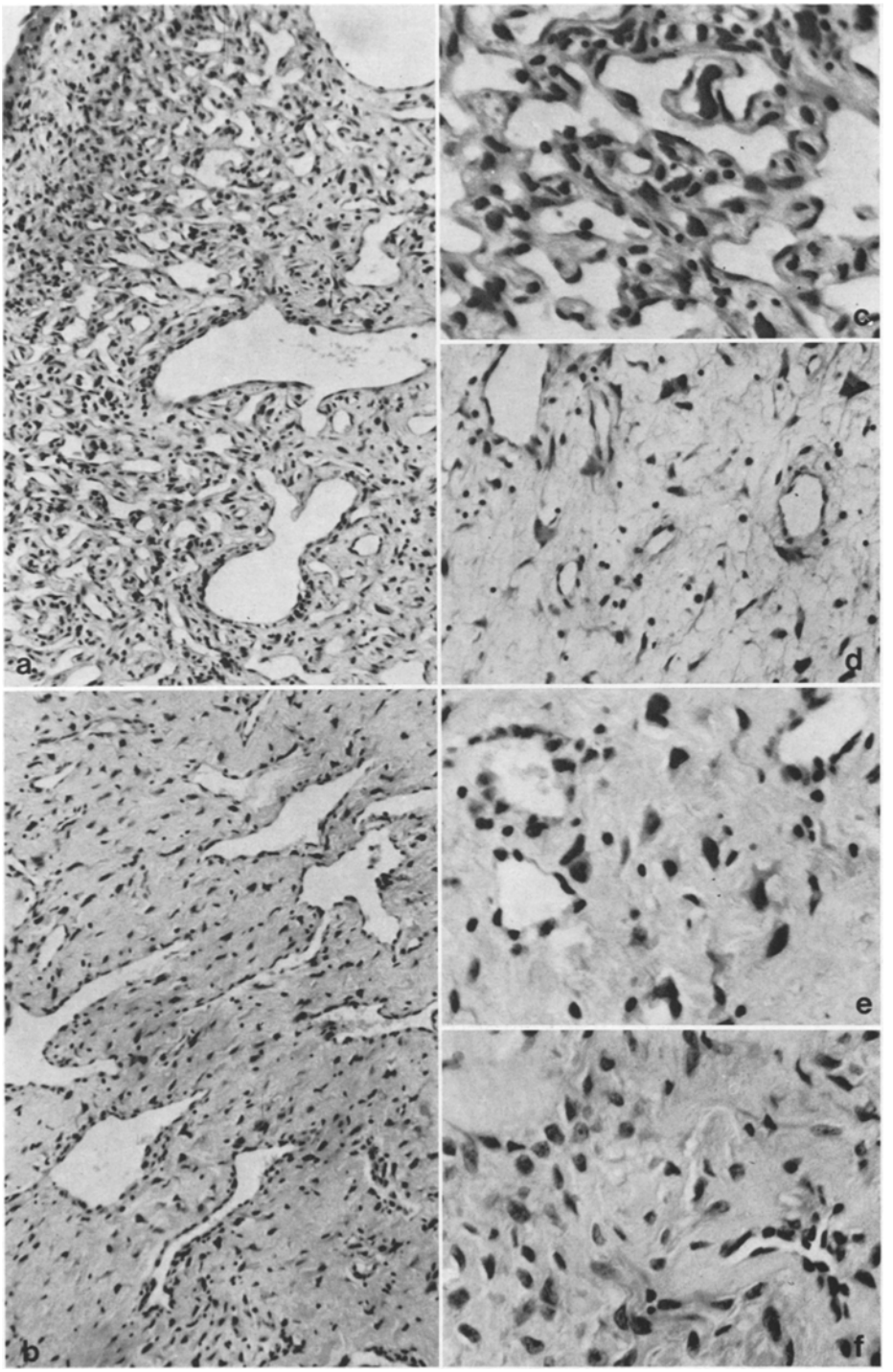

Fig. 1a—f. Light-microscopic pictures of nasopharyngeal angiofibromas demonstrating varying content of vessels which are partially lacunar (a, b) and behavior of intervascular stroma

The vessels are strikingly large and thin-walled, occasionally lacunar and without elastic fibers. In some vessels smooth muscle cells are present in a discontinuous layer, but mostly they are lacking. Occasionally the vessels give evidence of vasculitis or of thrombosis, and collections of lymphocytes and plasma cells are to be seen.

Electron microscopically three main cellular components can be distinguished:

1. The stromal cells which mostly resemble fibroblasts
2. Cells of the microvasculature
3. Inflammatory cells.

As a rule, the *fibroblasts* of the stroma are oblong to stellate-formed with long cytoplasmic processes. In correspondence with the different cell forms the nuclei reveal different patterns. Predominantly they are longish with numerous indentations and infoldings of their membranes (Fig. 2). Occasionally bizarre loops and projections of the nuclear membrane enclose cytoplasm and produce "nuclear blebs" and "pockets". Heterochromatin is regularly distributed and forms a clear dense zone at the nuclear margin. Some nuclei possess dense granules with clear halos (Fig. 2). The diameters of these round granules range from 200 to 3,000 Å. These phenomena were always encountered in connection with each other. Sometimes typical nuclear bodies mostly of types II or III after Bouteille et al. (1967), and large nucleoli were found. Distinct nuclear pores were often noticed, too.

The organelle content of fibroblasts is differing and makes possible the following subdivisions:

1. The classical fibroblast contains rough endoplasmic reticulum (RER) in abundance. The RER is often distended and forms sacs and cisterns (Fig. 2). A prominent Golgi apparatus, sometimes multicentric, a moderate content of mitochondria with oval, round, or branched bizarre forms, and many smooth-surfaced vesicles are visible. Sometimes a fibroblast shows a row of subplasmalemmal vesicles, which suggests pinocytosis or secretion. Loosely arranged filaments of about 100 Å in diameter must be mentioned, which are encountered in wavy bundles. In some cells filaments of 40–60 Å in diameter arranged as a fine filamentous network in subplasmalemmal position were observed as well as microtubuli of 240 Å in diameter.

2. In some cells the RER is reduced, the number of mitochondria and vesicles is increased, and the Golgi zone is very prominent and multicentric. There is an abundance of intracytoplasmic and subplasmalemmal vesicles. Several lysosome-like dense bodies, scattered lipid droplets, occasional membrane-bound collagen fiber fragments, and a discontinuous basement membranelike material on the outer cell surface especially in proximity of "pinocytotic" vesicles can be seen (Fig. 3). The stromal fibroblasts mentioned above have nuclei with "blebs" and "pockets" as well as intranuclear dense granules.

(c–f). In (a) and (c) proliferating capillaries with sparse intervascular stroma are seen, (d) shows a loosely textured stroma with myxoid appearance, in (b) and (e) a sclerosed stroma with fibroblastlike stromal cells and deposited collagen fibers is visible, and in (f) perivascular hyalinization is demonstrated (H&E; a: ×150; b: ×150; c: ×300; d: ×200; e and f: ×300)

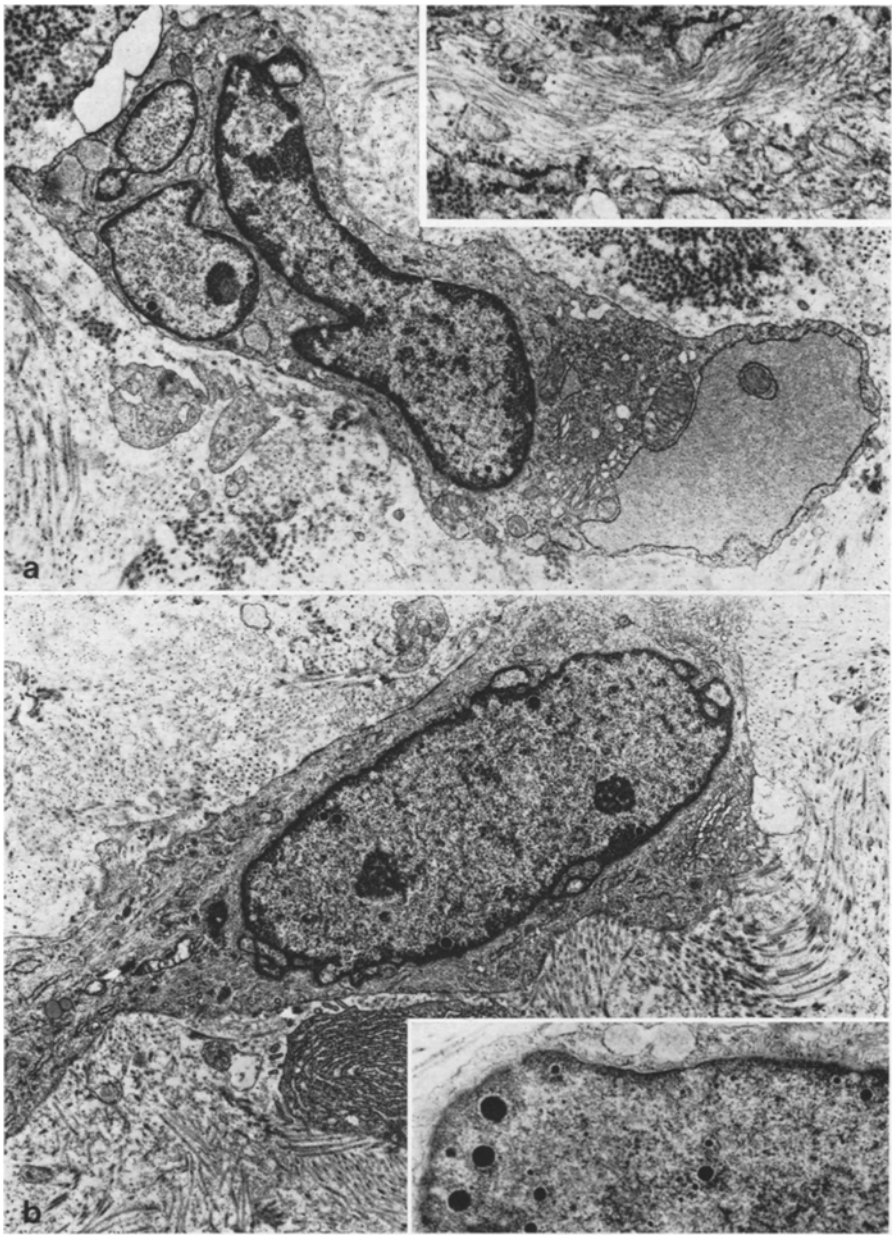

Fig. 2a and b. Stromal fibroblasts with predominating rough endoplasmic reticulum corresponding to classical fibroblasts. (a) Regionally extreme dilation of tubes of rough endoplasmic reticulum is striking. Nucleus lobulated imitating a multinucleated cell (×8,500). Inset: intracytoplasmic fibrils with diameter of 100 A, thought to be part of so-called cytoskeleton (×27,900). (b) Fibroblast with well-developed cytoplasmic organelles and nucleus which shows projections and infoldings of nuclear membrane resulting in formation of nuclear "blebs". Besides, dense intranuclear granules are to be observed (×6,800). Inset: detail of nucleus with several dense intranuclear granules (×16,100)

3. Fibroblasts with myoid differentiations (so-called myoid fibroblasts). The organelles resemble those of fibroblasts. Filaments with a diameter of 40–80 Å characterize this cell type. In some cells there are only subplasmalemmal bundles of filaments, while in some other cells they completely fill up the cytoplasm by displacing the other organelles. Within the bundles plasmalemmal densities and dense zones can be seen. A basement membranelike material on the outer cell surface and numerous caveolae increase the similarity to smooth muscle cells (Fig. 3). Occasionally these cells may develop into true smooth muscle cells via the intermediate stages mentioned above.

The *vessels* (Fig. 4) are predominantly of a capillary type. Only some of them correspond to postcapillary venules. The cells of the vessels proliferate, which results in different patterns. *Endothelial cells* are highly activated demonstrating numerous microvilli on the luminal side, heavy pinocytosis, and many smooth-surfaced vesicles. The Golgi apparatus is well developed. Filaments of 100 Å in diameter, some glycogen particles, and a moderate amount of mitochondria, free ribosomes, and tubes of RER as well as single multivesiculated bodies are to be noticed. The nuclei are longish and deeply indented. The heterochromatin is marginally condensed.

The *pericytes* surrounded by regular basement membranes show varying shapes ranging from oblong forms to bizarre stellate configurations. Many of them are connected by opposed plasma membranes. They are encountered close by the endothelial cells and at some distance from the vascular walls. The nuclei are also oblong and indented. In the cytoplasm the content of organelles is variable. Partially the organelle composition is almost identical with that of fibroblast. In other cells marked pinocytosis and filament bundles are found. Finally, the pericytes can be poor in organelles exhibiting only ribosomes, scattered microfilaments, some tubes of RER, and mitochondria. All cells of the vasculature never show the dense intranuclear granules of stromal fibroblasts.

The endothelial cells have interlocking junctions and are enveloped by basement membranes. If the basement membrane is absent the plasma membrane of a periendothelial cell forms an intercellular junction with the endothelial cell (simple apposition). Occasionally periendothelial cells cannot be distinguished from endothelial cells. They may have developed from proliferating and dividing endothelial cells.

Undifferentiated cells with only little plasma may be located within the vascular walls or amid the stromal cells (Fig. 4). Their nuclei are round, oval, or bean-shaped. There are only few cytoplasmic organelles consisting of some mitochondria and some filaments with a diameter of 80 Å as well as occasional tubes of RER. They may reveal some short fingerlike villi of their cell membrane and possess many caveolaelike structures. In the latter case they are localized within a vessel wall or in its proximity.

Discussion

Electron microscopically nasopharyngeal angiofibromas reveal proliferating cells of the stroma and of the vasculature. The stromal cells are generally fibroblastic. Some variations of the organelle composition can be distinguished as is well known from other examples of proliferating fibroblastic tissues:

(1) Activated "classical" fibroblasts, (2) activated fibroblasts with resemblance to histiocytelike cells due to subplasmalemmal vesicles and lysosomal

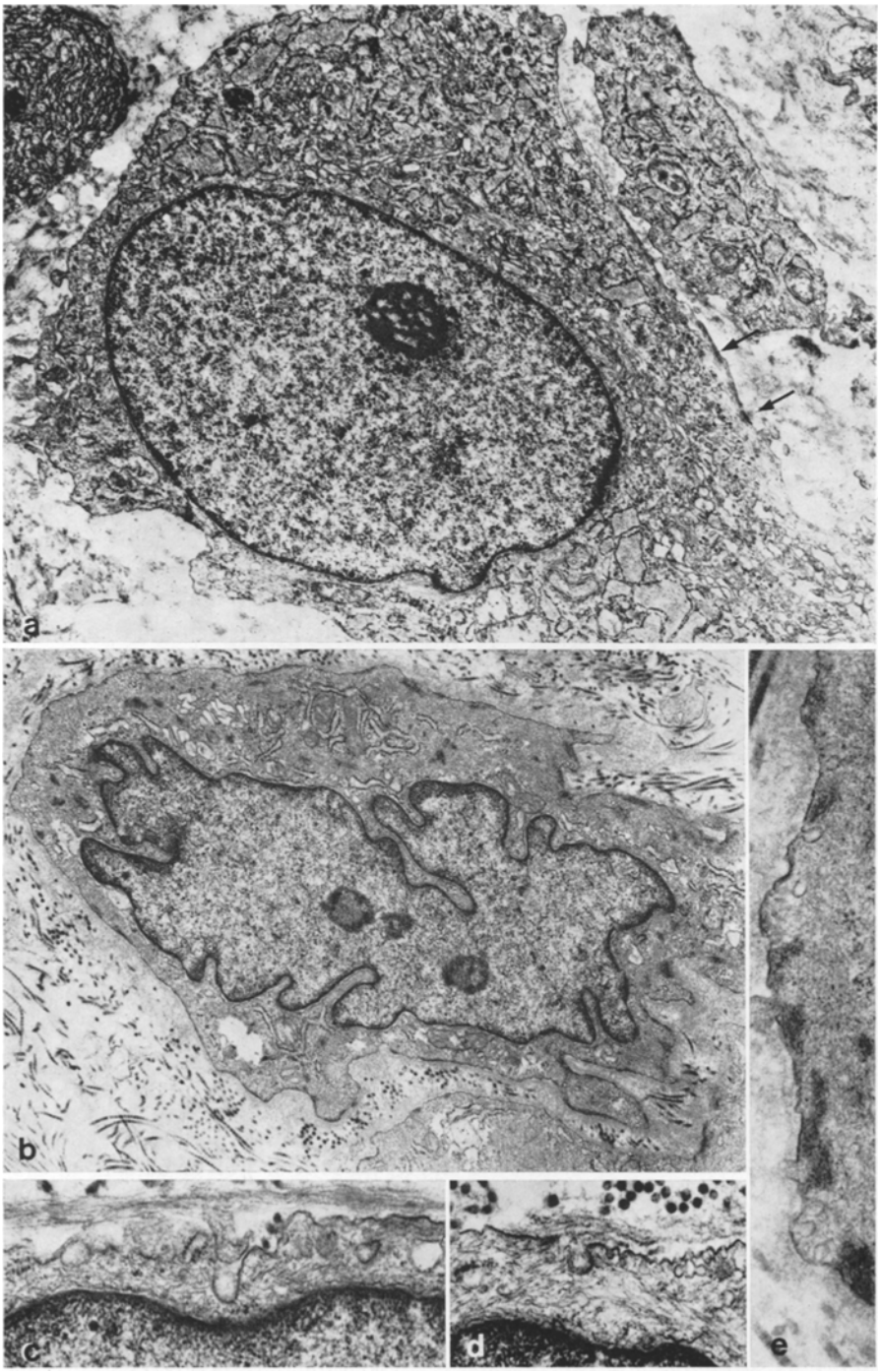

Fig. 3a—e. Modulations of fibroblasts. (a) Fibroblast with abundant organelles such as hypertrophic and multicentric Golgi apparatus and numerous vesicles, somewhat reduced rough endoplasmic reticulum, and single lysosomes. Furthermore subplasmalemmal microfilaments are visible (→) (×7,600). (b) Fibroblast with myoid appearance. Cytoplasm contains abundant microfilaments which resemble myofilaments in their densities and attachment

bodies, and (3) fibroblasts with myoid differentiations (cf. Katenkamp et al., 1976).

The term fibroblast with histiocytelike features does not necessarily imply a histiocytic function. The basement membranelike material close by the "pinocytotic" vesicles suggests a secretion rather than a resorption.

The morphologic spectrum of myoid fibroblasts ranges from typical myofibroblasts (see Gabbiani et al., 1971, 1972) to cells indistinguishable from smooth muscle cells. This is not surprising because of the close relationship between fibroblasts normally containing actin and myosin (Ishikawa et al., 1969; Lazarides and Weber, 1974; Gröschel-Stewart et al., 1975; Lazarides, 1975) and smooth muscle cells (Haust and More, 1966; Ross and Klebanoff, 1967; Moss and Benditt, 1970; Stiller and Katenkamp, 1976).

Certain variations in the structure of fibroblasts were repeatedly described in the literature. They are dependent on growth processes of fibroblasts in culture (Cireli, 1970; Comings and Okada, 1970; Lucky et al., 1975) or on functional stages of fibroblastic cells in the tissue (Raimondi and Beckman, 1967).

The nuclei of some stromal fibroblasts are characterized by two pecularities: (1) projections of nuclear membranes which form nuclear "blebs" and "pockets" and (2) intranuclear dense granules. Similar nuclear configurations have been described in blood cells (Smith and O'Hara, 1967), malignant lymphomas (Achong and Epstein, 1966; Dorfman, 1967), dermatofibromas (Katenkamp and Stiller, 1975), and dermatofibrosarcoma protuberans (Hashimoto et al., 1974; Auböck, 1976). Hashimoto and coworkers suggested that this nuclear configuration is a typical feature of dermatofibrosarcoma protuberans.

The intranuclear dense granules were not found in other lesions according to our knowledge. They are thought to be a distinctive and characteristic feature of stromal fibroblasts. Morphologically, they strongly resemble perichromatin granules (McGavran et al., 1969; cf. also Watson, 1962; Nicander, 1964; Bouteille, 1972) but they are considerably larger. Examination by ultrahistochemical methods demonstrated their proteinaceous nature (Küttner, 1972, 1973).

These typical nuclei are always associated with an organelle composition in the cytoplasm which characterizes the cells as classical fibroblasts or fibroblasts with histiocytelike features.

These characteristic nuclear structures of the stromal fibroblasts are connected with activated metabolism which is supported by the regular association with large nucleoli and nuclear bodies. Both the structures give evidence of nuclear hyperactivity (Yasozumi et al., 1975).

Nasopharyngeal angiofibromas are lesions with proliferating vascular and stromal components. Therefore, stromal cells, which are identified as fibroblasts with certain variations, constitute only a part of the growth. The other part of

sites. Besides, pinocytosis, moderate content of rough endoplasmic reticulum and few mitochondria present. Note deeply indented nucleus as morphologic correlate of cellular contraction (×10,800). (c) and (d) Cellular periphery with subplasmalemmal vesicles and a fibrillar material in the extracellular space. This must be interpreted as synthesis (c and d ×21,700).

(e) Subplasmalemmal fibrillar network and typical attachment sites (×31,000)

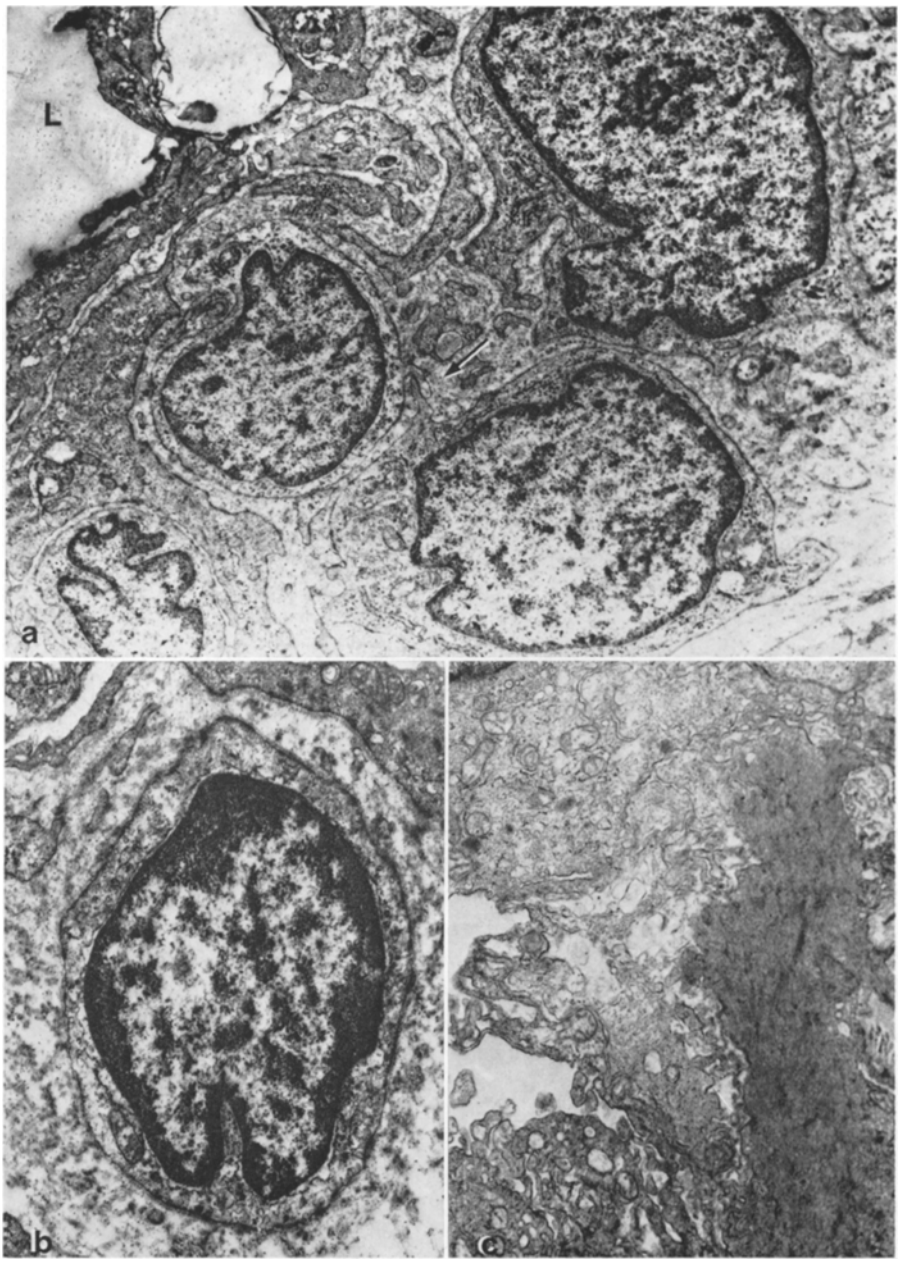

Fig. 4a—c. Proliferating capillary vessels in nasopharyngeal angiofibroma. (a) In close proximity of vascular lumen (*L*) several cells with slightly indented nuclei and narrow rim of cytoplasm can be seen. Cytoplasm of these cells only contains few organelles. Partial envelopment by basement membrane. Note intercellular contact (→) mediated by short cellular processes (×7,600). (b) "Undifferentiated" cell with sparse cytoplasm equipped with few mitochondria and short tubes of rough endoplasmic reticulum. Cell is surrounded by basement membrane. Furthermore, pinocytosis is noteworthy (×10,800). (c) Activated endothelial cells with abundant mitochondria and vesicles. Besides myoid differentiated cell in pericytic position visible (×11,500)

cells is contributed by proliferating vascular cells. Generally, our electron-microscopic results suggest rather reactive and hyperplastic lesions—a pseudotumorous lesion—than a true neoplasm (cf. Härmä, 1959; Pimpinella, 1964; Thomsen, 1971) and a classification as fibromatosis should be reconsidered.

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Doz. Dr. sc. Dankwart Stiller
Friedrich-Schiller-University
Department of Medicine
Institute of Pathology
Ziegelmühlenweg 1
DDR-69 Jena
German Democratic Republic