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Department of Pathology, University of Munich, Munich, West Germany (Chairman: Prof. Dr. M. Eder)

Palmar Fibromatosis-"Dupuytren's Contracture" A Comparison of Light Electron and Immunofluorescence Microscopic Findings

P. MEISTER, J. M. GOKEL, and K. REMBERGER

Summary

A comparison of light, immunofluorescence and electron microscopic findings in palmar fibromatosis (Dupuytren's disease) revealed that the three morphologic phase of Luck: I. proliferative, 2. involutional, and 3. residual, corresponded for the most part to I. fibroblastic, 2. myofibroblastic or 3. fibrocytic lesions, respectively. The spectrum between proliferative phase and residual phase appeared immunohistochemically as a decrease in collagen type III, and an increase of collagen type I. Myosin was found in substantial quantities only during the involutional phase, distinguished by myofibroblasts, presumably with contractile capabilities.

Thus, the clinical presence of contractures with palmar fibromatosis might also be explained in the absence of distinct fiber formation.

Introduction

Ledderhose (1920), whose name is associated with plantar fibromatosis, deserves credit for having described various cellular and fibrillar densities in palmar fibromatosis histologically. Luck (1959) recommended a subdivision of palmar fibromatosis into three histopathologic phases, according to different degrees of cellularity and fiber formation. In this review, we shall attempt to correlate A) light, B) electron and C) immunofluorescence microscopic findings with the different morphologic phases of palmar fibromatosis, or Dupuytren's disease (Meister et al., 1976; Gokel et al., 1976; Gokel and Hübner, 1977a; Meister et al., 1978).

Material and Methods

A) Light microscopy: In a series of 199 consecutive patients with palmar fibromatosis, the number of fibromatotic foci within the resected aponeurotic specimen was registered,

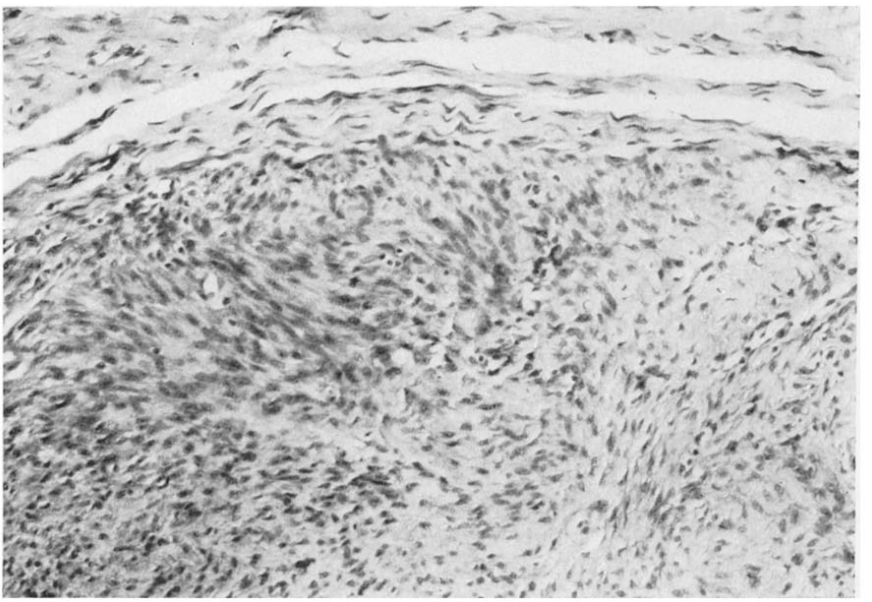

Fig. 1 a. Proliferative phase: cellular fibroblastic nodule, showing only slight focal and irregular fiber formation. HE; × 120.

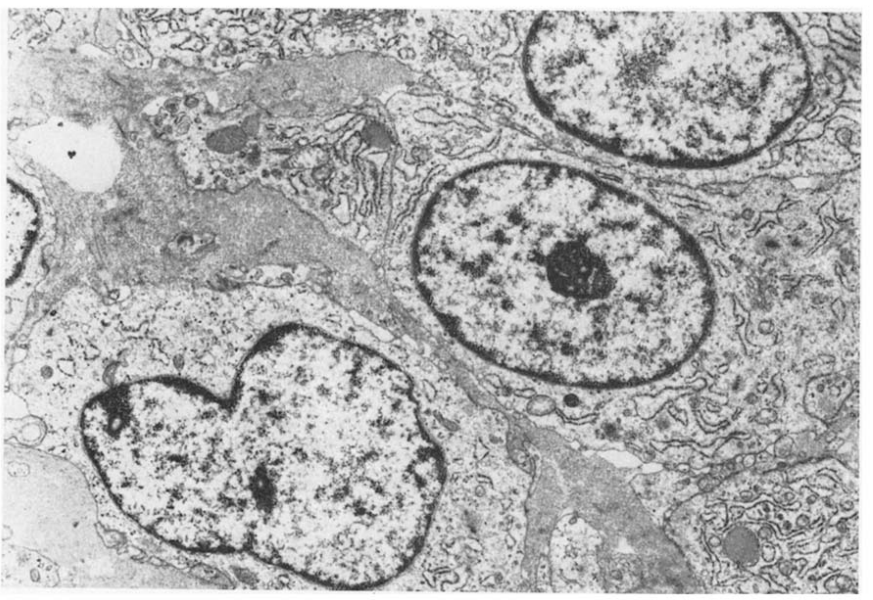

Fig. 1 b. Fibroblast-like cells with oval nuclei and prominent nucleoli. Well developed ergastoplasm, denoting a high metabolic activity (Arch. 12556). × 7,000.

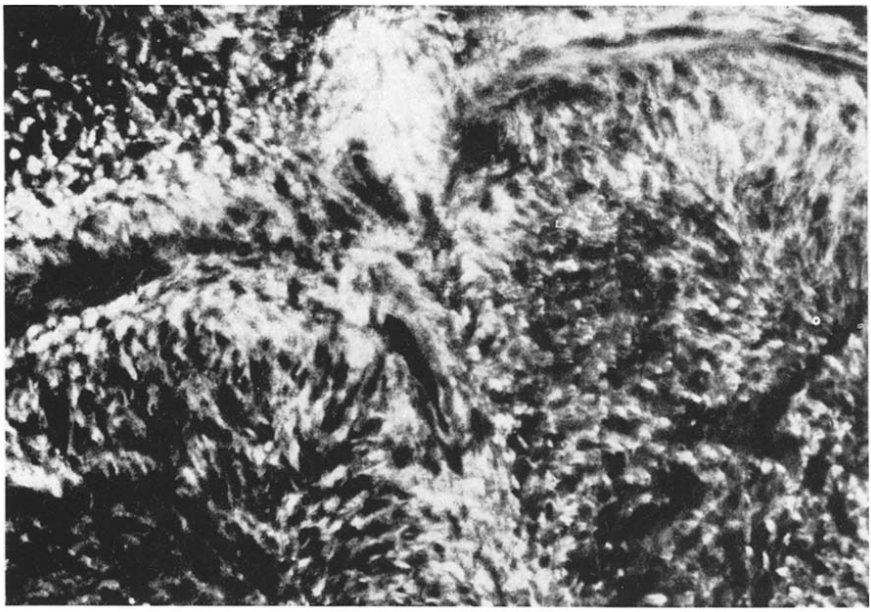

Fig. 1 c. Two proliferating nodules, with the majority of cells showing positive reaction with antibodies to collagen type III. Frozen section, indirect immunofluorescence technique; × 120.

and the morphologic phase was classified as either: 1. proliferative, 2. involutional or 3. residual. In addition to hematoxylin-eosin sections, van Gieson stains and silver impregnations served for evaluation of the fiber content (Meister et al., 1978).

B) Electron microscopy: In 21 surgical specimens, 1 mm^3 sized tissue cubes were fixed immediately in buffered glutaraldehyde and embedded in epon conform to standard procedures. From thin sections stained with Azure II-methylene blue, at least six representative areas for each of the 3 phases of palmar fibromatosis were selected for fine structural analysis, cut with diamond knives, stained with uranyl acetate and lead citrate, and examined in a Philips EM 300 (Gokel and Hübner, 1977a).

C) Immunofluorescence microscopy: In 84 cases, the different collagen types were studied by the indirect immunofluorescence technique (Remberger et al., to be published), using type-specific antibodies against collagen types I, II, and III (Gay et al., 1976; Gay and Miller, 1978). In addition, in 45 cases we attempted to mark intracellular myosin immunohistochemically using antibodies to myosin of chicken stomach (Gröschel-Stewart et al., 1976).

Results

In 90% of the 199 patients examined, multiple foci of fibromatosis within the resected specimen of the palmar aponeurosis were recognizable macroscopically and microscopically. 193 patients could be classified by

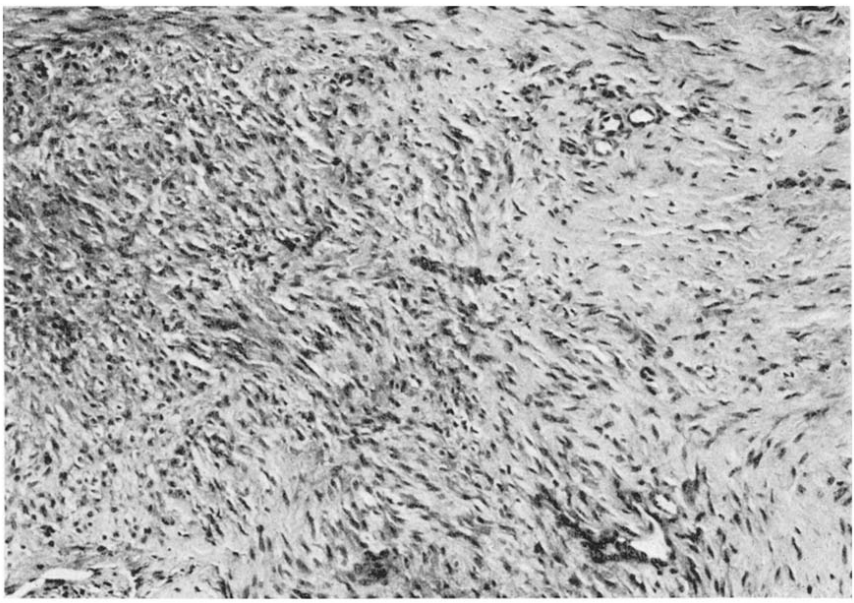

Fig. 2 a. Involutional phase: cellular focus with distinct fiber formation and beginning parallel alignment. HE; × 120.

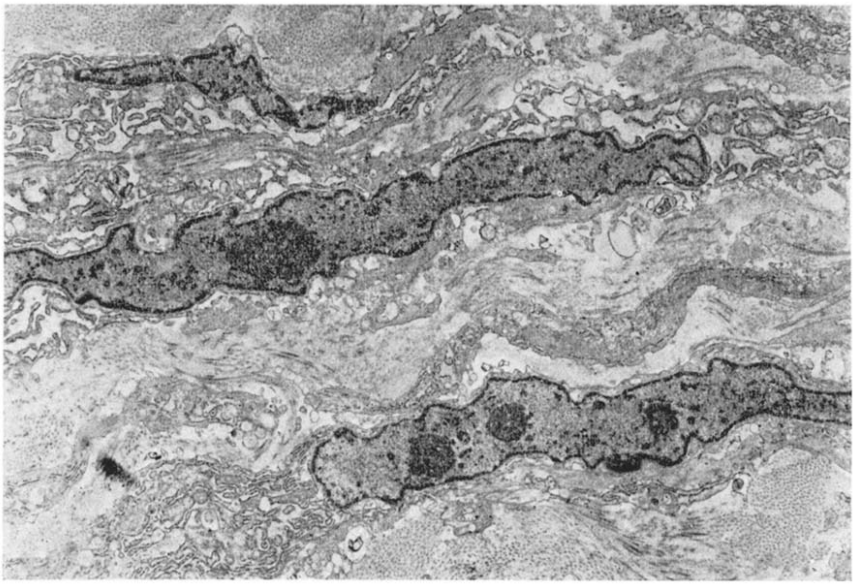

Fig. 2 b. Myofibroblasts with indented nuclei showing irregulary distributed chromatin. Well developed ergastoplasm and small bundles of filaments within the cytoplasm. Extracellularly: oriented collagen bundles (Arch. 12002). × 5,000.

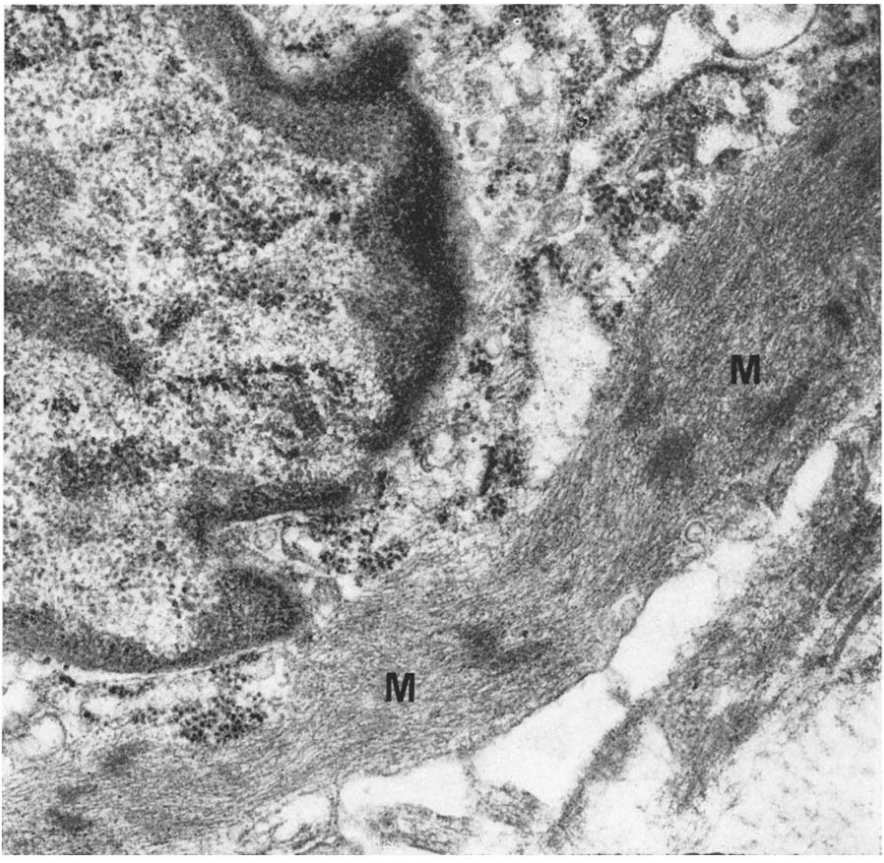

Fig. 2 c. Periphery of myofibroblast: bundles of microfilaments ("myofilaments" = M) below and parallel to the cell membrane, with irregulary interspersed, electron dense areas (Arch. 9889). × 43,000.

the predominant type of individual foci of fibromatoisis as follows: 1. 79 patients as proliferative phase, 2. 82 patients as involutional phase, and 3. 32 patients as residual phase.

No foci of palmar fibromatosis were found in routine sections taken from the resected aponeurosis in only 6 patients (3%).

Comparison of A) light, B) electron and C) immunofluorescence microscopic findings

1. The proliferative phase revealed: A) highly cellular foci with oval nuclei, chiefly reticulin fibers, and only a few fuchsinophilic fibers. There was no special alignment of the cells. Blood vessels were numerous (Fig.

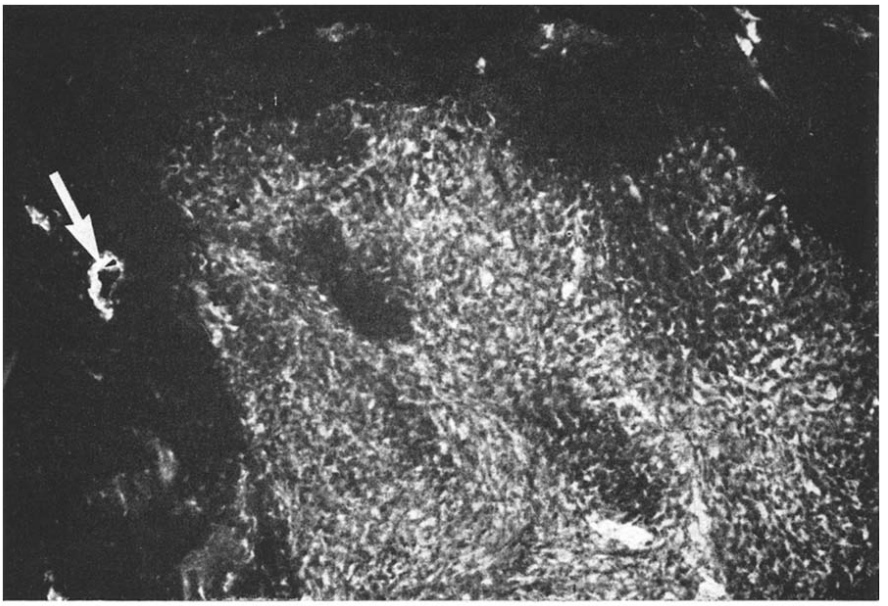

Fig. 2 d. Myofibroblasts marked with antibodies to myosin. Strongly positive reaction in vascular smooth muscle cells (→). Frozen section, indirect immunofluorescence technique; × 120.

1 a). B) Electron microscopically, the majority of cells were plump with oval nuclei, smooth nuclear membrane, prominent nucleoli, and a well-developed rough endoplasmic reticulum with prominent cisternae containing electron-lucent material (Fig. 1 b). Intracytoplasmic microfilaments were rare. In the interstices, there were scattered, unaligned fibers showing the periodicity of collagen. Occasionally, intracytoplasmic collagen compatible with "long spacing collagen" could also be seen. The majority of cells were compatible with fibroblasts. C) Immunohistochemically, we demonstrated chiefly collagen type III and a little collagen type I (Fig. 1 c). Cells with a positive reaction for myosin were scant.

2. The involutional phase showed A) elongation of nuclei of lesional cells and an increase of fuchsinophilic fibers (Fig. 2 a). B) The fine structure was distinghished by cells with indented nuclei, irregulary distributed chromatin, prominent nucleoli, and well developed rough endoplasmic reticulum with cisternae characteristic of fibroblasts. There were a few pinocytotic vesicles beneath the cell surface. The surface was covered by basement membrane-like material. Intercellular junctions were not encountered. Large bundles of collagen were present extracellulary with evidence of

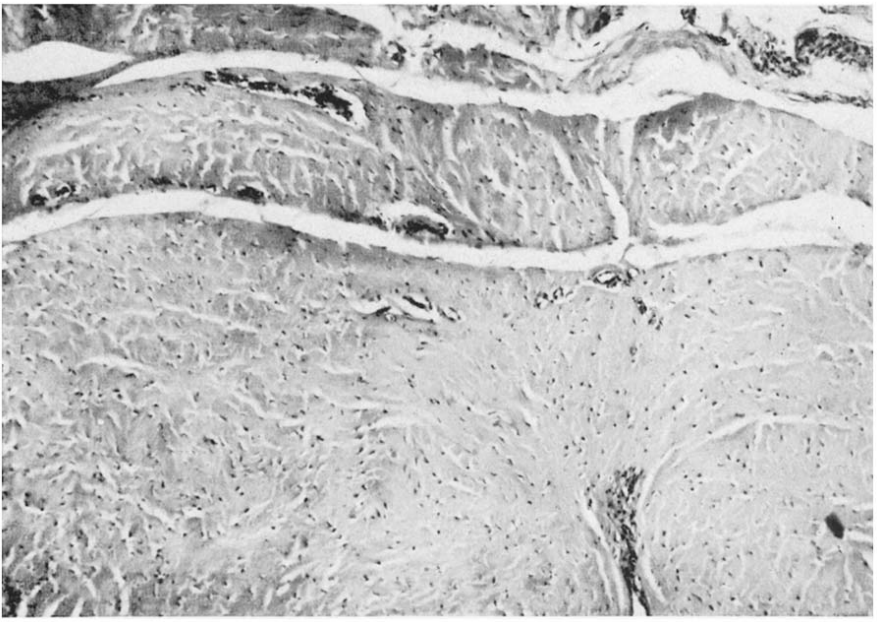

Fig. 3 a. Residual phase: nodule with decreased cellularity and hyalinization (upper third: intact aponeurosis). HE; × 120.

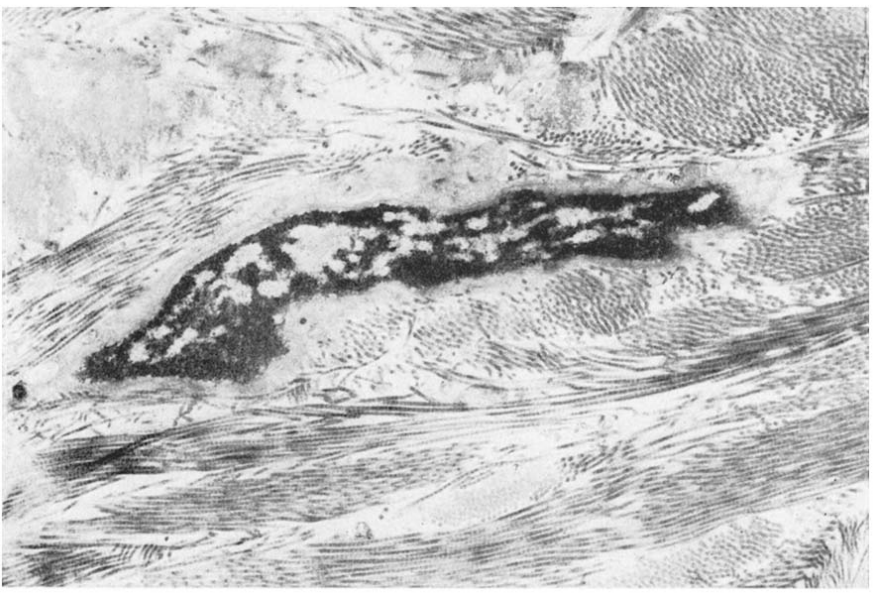

Fig. 3 b. Elongated, electron dense nucleus with narrow cytoplasmic rim, squeezed between broad bundles of densely packed extracellular collagen fibers (Arch. 20504). × 12,300.

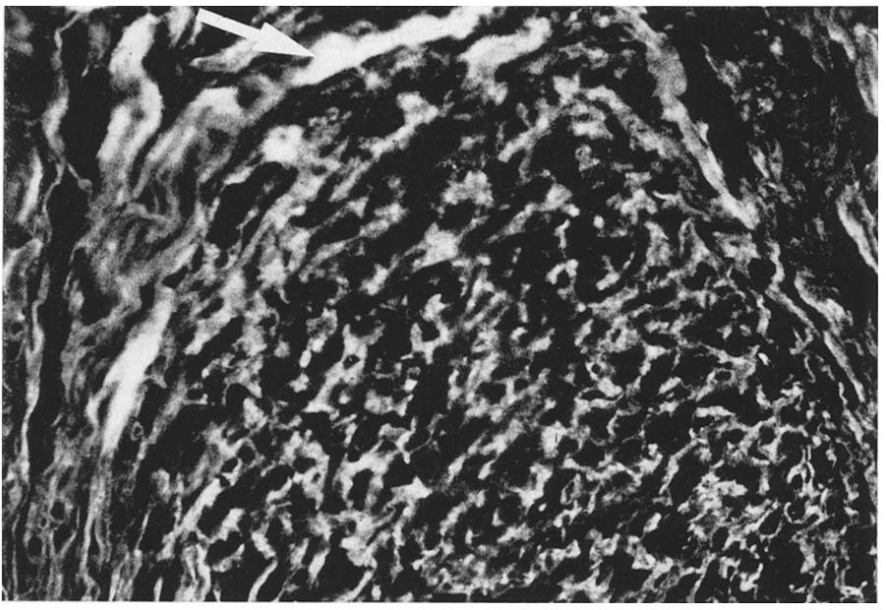

Fig. 3 c. Nodule showing a positive reaction with antibodies to collagen type I, which is found in broad extracellular deposits (→) in the adjacent aponeurosis. Frozen section, indirect immunofluorescence technique; × 120.

aggregation. Packed microfilaments (5–8 nm in diameter) beneath the cell membrane and parallel to the long axis of the cell were typical. These microfilaments are compatible with myofilaments, and the cells qualify as myofibroblasts (Fig. 2 b and c). C) Immunohistochemically, an increase of collagen type I was found at the expense of type III. The presence of myosin was impressive in a substantial number of cells, as shown by the indirect immunofluorescence technique (Fig. 2 d).

3. The residual phase showed A) distinct nodules, which, however, were not distinguished from the surrounding aponeurotic tissue by a higher cellular density. Some lesions were hyalinized with decreased cellularity (Fig. 3 a). B) On electron microscopy, the nuclei were more elongated and electron dense (hyperchromatic). The cytoplasm contained few organelles. Cisternae of the rough endoplasmic reticulum and myofilaments were not observed. The cells were trapped between bundles of collagen (Fig. 3 b and c). They qualified as fibrocytes. C) In this phase, there was an overwhelming predominance of collagen type I (Fig. 3 d). In 4 cases, collagen type II ("cartilage collagen") was found as well.

Discussion

We were not able to demonstrate any degenerative changes within the connective tissue, prior to or during the phase of cellular proliferation, with the methods applied (Millesi, 1970; Nemetschek et al., 1976).

Our findings suggest that palmar fibromatosis begins with a repair process. In this regard siderophages, which were occasionally but not always observed, should be mentioned, suggesting previous bleeding and a possible triggering trauma and/or vascular lesion. There are primitive mesenchymal cells with many capillaries in the proliferative phase, comparable to fibroblasts in cell cultures or granulation tissue at 2 days of age (Remberger and Hübner, to be published). Because of their characteristic vascular relations, the question of the pericytic nature of the proliferating cells has been raised (Katenkamp and Stiller, 1976). Possible regional differences with centrifugal maturation of the proliferating cells, could not yet be proven by examining a sufficient number of sections (Gokel, unpublished data). A substantial number of cells qualified as fibroblasts. These cells occasionally contained a peculiar collagen ("fibrous long spacing collagen"), the origin of which is not fully understood (Gokel and Hübner, 1977b).

During the involutional phase, as in granulation-tissue at 5 to 10 days of age or in comparable cell cultures, there is a quantitative increase of collagen production and qualitatively an increase of collagen I, which shows fuchsinophilia (Meister et al., 1977; Remberger et al., 1977). In addition, quite a number of cells revealed bundles of myofilaments and additional fine structural features, qualifying them as "myofilaments" (Feiner and Kaye, 1976; Gabbiani et al., 1973; Gabbiani et al., 1976; Gokel and Hübner, 1977; Ryan et al., 1974). In the residual phase, we found predominantly mature fibrocytes with abundant bundles of extracellular collagen, chiefly of type I but occasionally of type II ("cartilage collagen"), with light microscopically recognizable hyalinization, as in scar tissue.

The following pathogenetic concept of palmar fibromatosis and contracture is suggested: 1. initiation chiefly by fibroblastic proliferation, 2. contracture by contractile cells ("myofibroblasts"), 3. establishment of the contracture by an increase in the number of collagen fibers and maturation of collagen (predominantly collagen type I) (Gabbiani and Majno, 1972; Meister et al., 1976). In the residual phase of palmar fibromatosis, there is not only a decrease in cell density with an increase of intercellular collagen light microscopically, but also a decrease and disappearance of myofilaments, that is a disappearance of myofibroblasts, seen at the level of fine structure. Comparison of the different morphologic phases of palmar fi-

bromatosis with presence or absence of clinical contracture revealed no significant differences. In other words, contractures had also been present in the absence of distinct collagen fiber formation, i.e. in the proliferative phase (Meister et al., 1978).

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Professor Dr. med. Peter Meister, Pathologisches Institut der Universität, Thalkirchner Str. 36, D-8000 München 2