

## **Palmar fibromatosis (Dupuytren's contracture)**

### **Ultrastructural and enzyme histochemical studies of 43 cases**

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**Summary.** Forty three cases of palmar fibromatosis were studied by light and electron microscopy, enzyme histochemistry, and ultrastructural immunohistochemistry. By electron microscopy most of the cells composing the nodules in both the proliferative and the involutinal stages were identical to myofibroblasts. The myofibroblasts in the involutinal nodules often possessed microfilament aggregates probably representing contraction of micro(actin)filaments in the cytoplasm. The proliferative nodules revealed small perivascular haemorrhages and haemosiderin deposits accompanied by accumulation of macrophages and some lymphocytes; these inflammatory cells possibly secrete a certain growth factor inducing proliferation of genetically abnormal fibroblasts and myofibroblasts. Diaminopeptidase IV was detected in myofibroblasts and fibroblasts by enzyme histochemistry and ultrastructural immunohistochemistry; the enzyme may play a role in the metabolism of intercellular substances. Some perivascular mesenchymal cells, interpreted as variants of myofibroblasts, had moderate activity of alkaline phosphatase.

**Key words:** Fibromatosis – Dupuytren's contracture – Myofibroblast – Ultrastructure – Histochemistry

### **Introduction**

Palmar fibromatosis (Dupuytren's disease) is characterized by a tumour-like proliferation of fibroblast-like cells producing nodules and cords in the palmar fascia (Allen 1977; Enzinger and Weiss 1983). Gabbiani and Majno (1972) demonstrated for the first time that the cells composing the nodules

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were myofibroblasts exhibiting three significant ultrastructural features: (1) a fibrillar system similar to that of smooth muscle cells; (2) nuclear deformations like those found in contracted cells; (3) cell-to-cell and cell-to-stroma attachments. Based on these data, Gabbiani and Majno suggested that contraction of myofibroblasts played a role in the pathogenesis of the contraction observed clinically. Since then, some ultrastructural and histochemical studies have been reported in the literature (Katenkamp and Stiller 1976; Gokel et al. 1976; Nemetschek et al. 1976; Meister et al. 1979; Müller et al. 1983; Iwasaki et al. 1983a), but little is known about the true nature and a etiology of the disease. To answer these questions, we have investigated fresh surgical material from 43 patients with palmar fibromatosis. Methods employed included light and electron microscopy, enzyme histochemistry, and ultrastructural immunohistochemistry.

### Materials and methods

During the period from September 1982 through March 1984, fresh surgical material from 43 patients with palmar fibromatosis was examined<sup>1</sup>. The clinicopathological features of these patients have been reported elsewhere (Müller et al. 1983). There were 27 males and 16 females who ranged in age from 25 to 73 years (median 53 years). The surgical specimens were immediately divided into four parts and processed for light and electron microscopy, enzyme histochemistry, and ultrastructural histochemistry.

*Light microscopy.* Paraffin sections from the material fixed in 4% buffered formaldehyde were stained with haematoxylin and eosin, Masson-Goldner trichrome, Giemsa, and elastica van Gieson. Semithin sections from araldite blocks for electron microscopy were stained with toluidine blue and utilized for light microscopy.

*Electron microscopy.* Portions of fresh surgical specimens were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4, postfixed in 1% buffered OsO<sub>4</sub>, dehydrated in acetone, and embedded in araldite (Serva, Heidelberg). The ultrathin sections were cut with a Reichert ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Siemens Elmiskop 101.

*Light microscopic enzyme histochemistry.* In each case light microscopic enzyme histochemical studies were carried out using frozen sections of fresh surgical materials. The enzymes investigated included diaminopeptidase IV (DAP IV, Lojda 1979), alkaline phosphatase (ALP, Burstone 1962), acid phosphatase (ACP, Barka and Anderson 1962), and  $\alpha$ -naphthyl acetate esterase (ANA-Est, Yam et al. 1971).

*Ultrastructural enzyme histochemistry for ALP.* Portions of fresh material were fixed in 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2 at 4°C for 2 h; some material was fixed in a mixture of 0.5% glutaraldehyde and 4% paraformaldehyde in the same buffer at 4°C for 1 h. After fixation, the tissue was washed in cacodylate buffer and sliced into 40  $\mu$ m sections with an Oxford Vibratome. For ALP reaction, the free-floating sections were incubated at 37°C for 1 h in the medium according to Mayahara et al. (1967). The sections were postfixed in 1% OsO<sub>4</sub>, dehydrated through graded acetone, and flat embedded in araldite. Ultrathin sections unstained or stained with lead citrate were used for electron microscopy.

In each case, control preparations were incubated in the medium without substrate.

<sup>1</sup> We thank Prof. Dr. Kootz, DRK-Krankenhaus Frankfurt/M. for providing the surgical material

*Ultrastructural immunohistochemistry.* Sections of 1  $\mu$ m thickness, immunostained with rabbit anti-mouse IgG against human DAP IV (Dr. Gabbiani, University of Kiel, Germany), and developed with diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) at room temperature (Owens 1976). The sections were mounted on slides, stained with toluidine blue. The sections were dipped in 1% lead citrate (Pierce and Warriner, MO, USA) dissolved in 0.1 M cacodylate buffer, then were placed in a 1% lead citrate solution and were post-fixed in 1% lead citrate.

### Results

*Light microscopy.* The lesions showed considerable variability in size and distribution based on the predilection for the palmar region (Fig. 1c), and the histopathological features. Nodules of basophilic material, small foci of proliferating cells (Fig. 1b) accompanied by mast cells.

Mainly in the nodules, the cytoplasm of the cells was stained with toluidine blue. The bodies measuring 1–2  $\mu$ m, comparable structures, and eosinophilic round bodies.

*Electron microscopy.* The immature fibroblasts (mature fibroblasts) were arranged in nodules (mature fibroblasts) with a small number of well-developed cytoplasmic complexes, and bundles of microfilaments. The bundles of microfilaments, were located within these bundles. Usually observable features of myofibroblasts and uncoated vesicles, varying at the basal lamina. Int

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*Ultrastructural immunohistochemistry for DAP IV.* Free floating tissue sections, 10 to 20  $\mu$ m in the thickness, were prepared by the same method as for ALP. A monoclonal mouse antibody against human DAP IV was supplied kindly by Prof. Parwaresch in the Institute of Pathology, the University of Kiel. Before immune reaction the sections were preincubated in 0.02% saponin (Sigma, St. Louis, MO, USA) in Dulbecco's phosphate buffered saline (PBS) for 30 min at room temperature (Ohtsuki et al. 1978; Tokumitsu et al. 1981). The sections were then incubated with the monoclonal anti-DAP IV antibody diluted 1:100 in PBS for 24 h at 4°C. The sections were washed 3 h in PBS and incubated overnight at 4°C in peroxidase-labeled rabbit anti-mouse IgG (Miles, Elkhart, IN, USA) diluted 1:5 in PBS. After washing in PBS the sections were dipped in a 0.05% 3,3'-diaminobenzidine-4HCl (DAB, Sigma, St. Louis, MO, USA) dissolved in 50 mM Tris buffer (PH 7.6) for 15 min at room temperature, and then were placed in a medium of 0.03% H<sub>2</sub>O<sub>2</sub> added to DAB solution for 15 min. The samples were post-fixed in 1% OsO<sub>4</sub> and processed for electron microscopy.

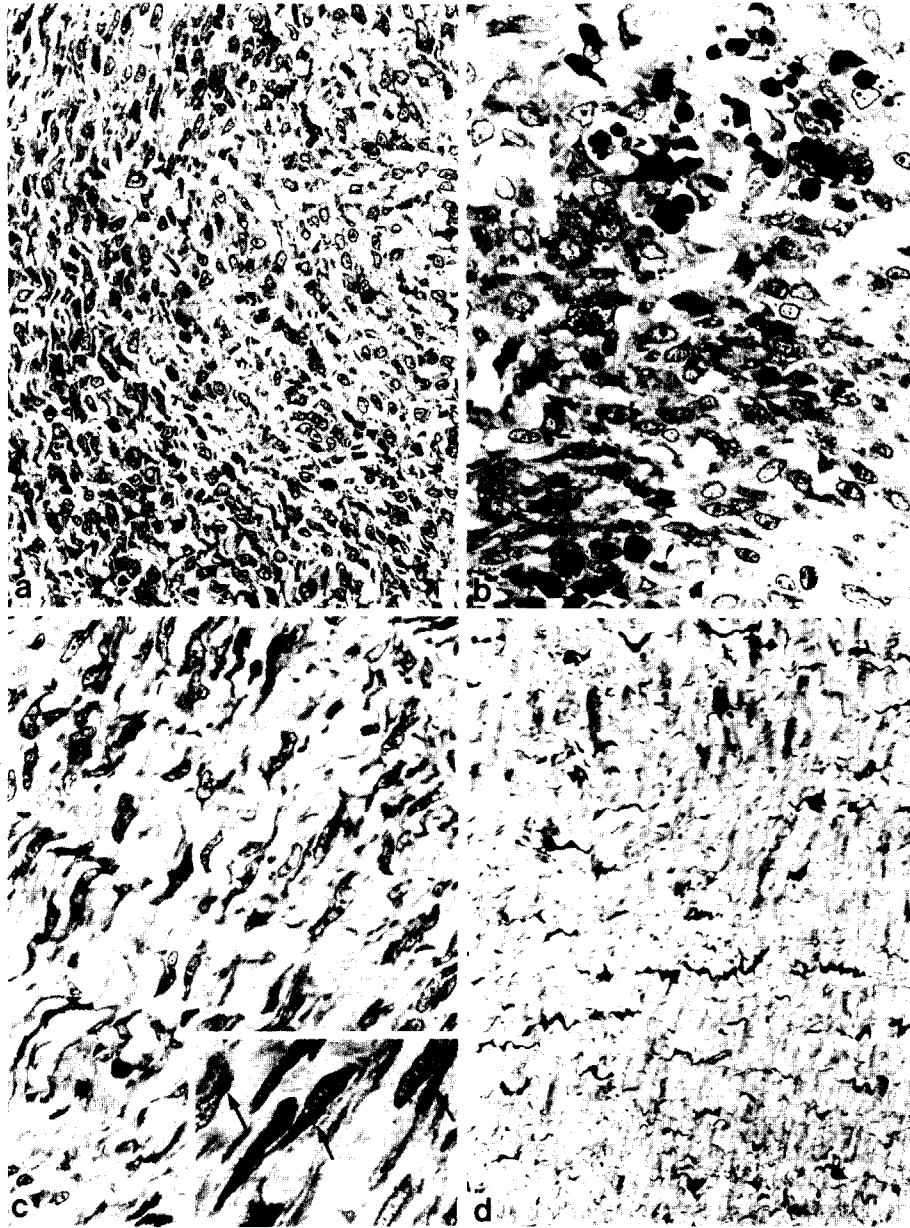
## Results

*Light microscopy.* Because many patients had multiple nodules showing considerable variation in their histological appearance, classification was based on the predominant histological picture. Of the 43 samples examined, 14 were in the proliferative stage (Fig. 1a), 20 were in the involutional stage (Fig. 1c), and 9 were in the residual stage (Fig. 1d), according to the histopathological classification of Luck (1959).

Nodules of both proliferative and involutional stages often contained small foci of perivascular haemorrhage and haemosiderin deposition (Fig. 1b) accompanied by accumulation of macrophages, some lymphocytes and mast cells.

Mainly in the involutional lesions, small round bodies were found in the cytoplasm of fibroblast-like cells (Fig. 1c inset). In semithin sections stained with toluidine blue, these structures were recognized as round blue bodies measuring 1 to 2  $\mu$ m in diameter, but it was very difficult to find comparable structures in paraffin sections, which rarely revealed eosinophilic round bodies in some cells.

*Electron microscopy.* Three main types of cells were identified in the lesions: immature fibroblasts (Fig. 2b), myofibroblasts (Figs. 2a, 4a), and fibrocytes (mature fibroblasts, Fig. 5). The lesions in the proliferative stage (proliferative nodules) were composed chiefly of myofibroblasts (Fig. 2a) admixed with a small number of immature fibroblasts (Fig. 2b). Myofibroblasts possessed well-developed rough endoplasmic reticulum (RER), prominent Golgi complexes, and bundles of microfilaments measuring 5–7 nm in diameter. The bundles of microfilaments, running parallel to the long axis of the cells, were located mainly in the cell cortex just under the cell membrane. Within these bundles were interspersed dense patches identical to those usually observable in the smooth muscle cells (Figs. 2a and 4a). Other features of myofibroblasts included multiple indentations of the nucleus, coated and uncoated vesicles, a moderate number of mitochondria, many polyosomes, varying amounts of 10 nm filaments, microtubules, and a few lysosomes. Frequently part of the cell surface was covered by discontinuous basal lamina. Intercellular junctions, chiefly of the intermediate type and



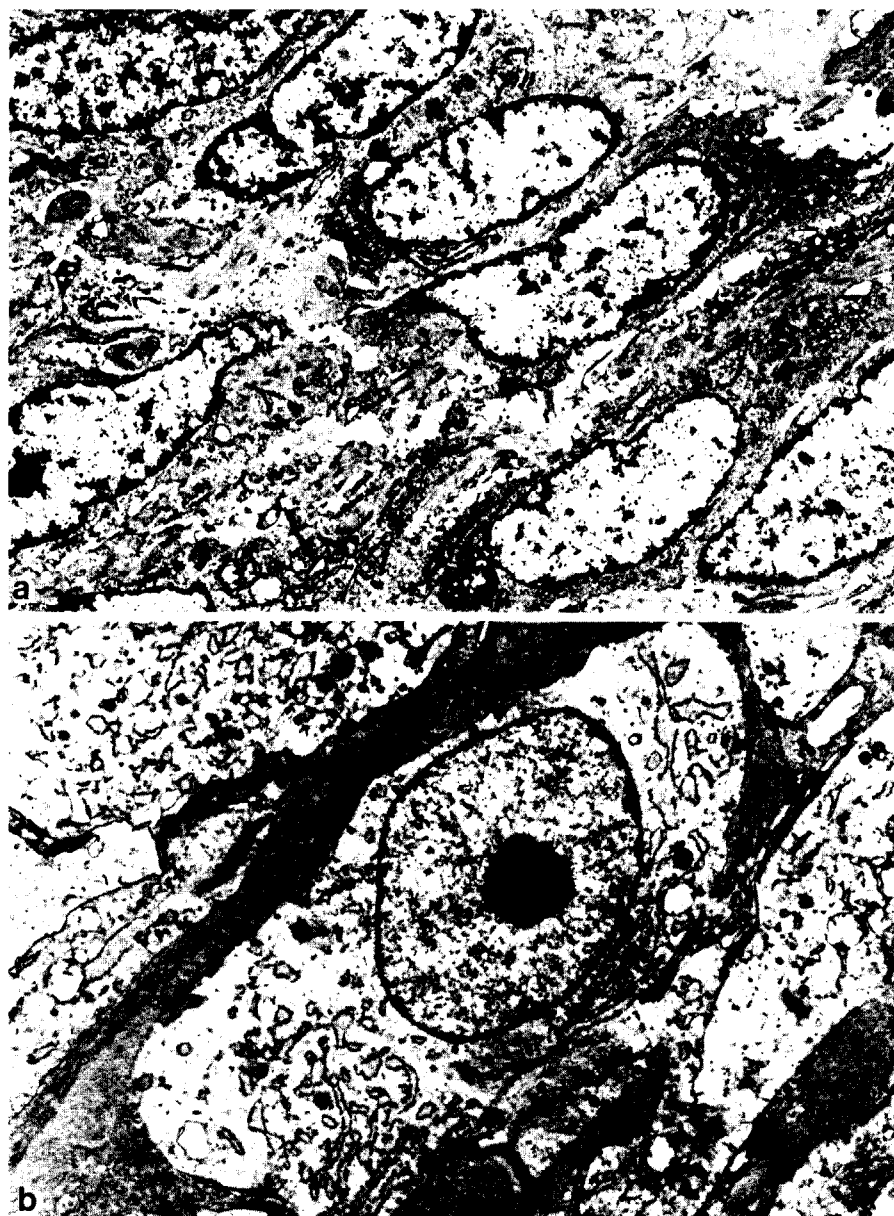
**Fig. 1.** Histological appearances of palmar fibromatosis. Semithin sections, toluidine blue stain: **a** Proliferative stage.  $\times 240$ . **b** Small foci of haemorrhage with accumulation of macrophages containing erythrocyte debris and haemosiderin. Notice a dense proliferation of fibroblast-like cells adjacent to the haemorrhagic focus.  $\times 390$ . **c** Involutonal stage. Elongated cells are separated by collagen fibers.  $\times 390$ . *Inset*: Small round bodies (*arrows*) are found in the cytoplasm of fibroblast-like cells.  $\times 530$ . **d** Residual stage showing a scar-like fibrous tissue composed of fibrocytes and abundant intercellular collagen.  $\times 240$



**Fig. 2.** Proliferative stage of palmar fibromatosis. Fibroblasts characterized by dense patches of condensed chromatin and a distinct cytoplasm.



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**Fig. 2.** Proliferative stage. **a** The majority of cells composing the proliferative nodule are myofibroblasts characterized by well-developed RER and bundles of microfilaments with interspersed dense patches.  $\times 5,700$ . **b** A young fibroblast having a large nucleus with evenly distributed chromatin and a prominent nucleolus.  $\times 7,500$

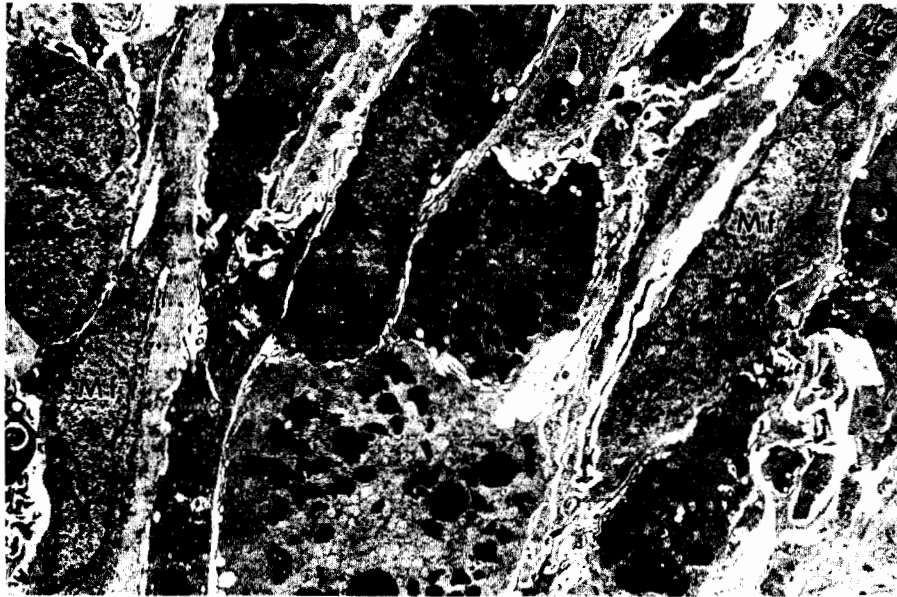


Fig. 3. A small focus of haemorrhage in the proliferative nodule. Many phagocytic macrophages contain erythrocyte debris and haemosiderin aggregates. Notice myofibroblasts (*Mf*) near to the macrophages.  $\times 4,200$

sometimes of the desmosome type (Fig. 4b), were noted between adjoining myofibroblasts. Cell-to-stroma junctions were not rarely encountered; they were associated with subplasmalemmal dense patches often connected with microfilament bundles.

In addition to myofibroblasts, the proliferative nodules contained some immature fibroblasts having a moderate amount of RER, many polysomes, a large nucleus with evenly distributed chromatin, and a prominent nucleolus (Fig. 2b). Transitional forms between immature fibroblasts and myofibroblasts were often present. The intercellular space was occupied by fine matrix fibrils and a granular substance, while mature collagen was scanty.

In the foci of perivascular haemorrhage, one could see infiltrates of many macrophages and some lymphocytes admixed with myofibroblasts and a few fibroblasts. These macrophages had numerous filopodia and abundant cytoplasm containing erythrocyte debris, granular aggregates of haemosiderin, and numerous residual bodies (Fig. 3).

The lesions of the involutinal stage consisted of nodules and fibrous cords extending from them. The nodules were composed almost exclusively of myofibroblasts embedded in a well-vascularized fibrous stroma (Fig. 4a). A few fibrocytes as well as intermediate forms between myofibroblasts and fibrocytes were also sometimes recognized. Corresponding to the small round bodies seen by light microscopy (Fig. 1c inset), the cytoplasm of myofibroblasts often contained small aggregates of microfilaments forming a dense meshwork, in which several dense patches were scattered (Figs. 4b, c). These aggregates, measuring 1–2  $\mu\text{m}$  in diameter, bore a close resem-



Fig. 4. Involutinal nodules with many dense patches are present in the perivascular haemorrhage. The desmosomes are noted between these



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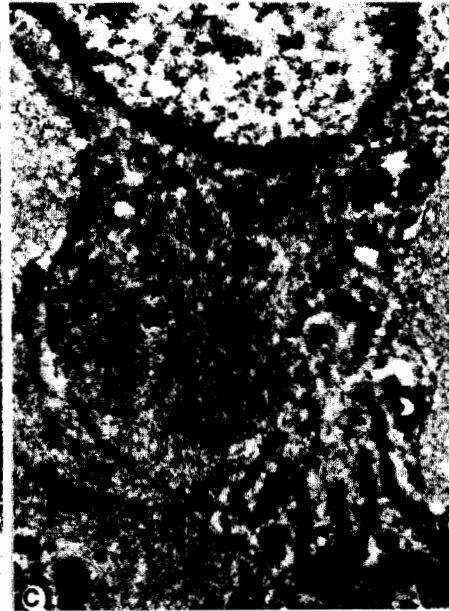
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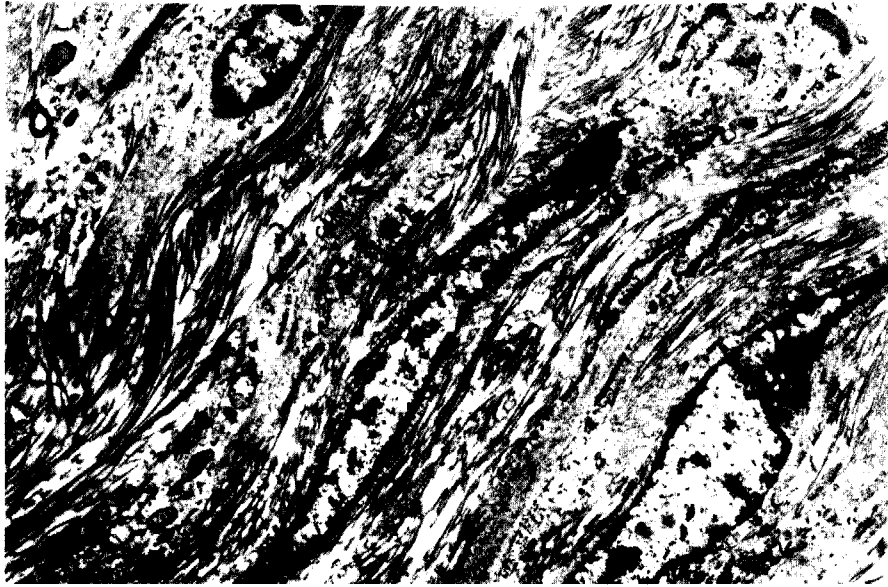


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**Fig. 4.** Involutional stage. **a** A typical myofibroblast showing cytoplasmic microfilament bundles with many dense patches.  $\times 11,000$ . **b** Microfilament aggregates containing dense patches are present in the periphery of cytoplasm of the adjoining cells. A desmosome-like structure is noted between these cells.  $\times 15,000$ . **c** Microfilament aggregate with dense patches.  $\times 18,600$



**Fig. 5.** A residual stage lesion consisting of slender fibrocytes embedded in a collagenous matrix.  $\times 7,000$

blance to the contracted form of actin filament bundles found in cultured myofibroblasts (Iwasaki et al. 1983 b). Although the aggregates of microfilaments were most common in the involutinal nodules, they were sometimes noted also in the proliferative nodules.

The intercellular matrix of the involutinal nodules revealed a fine granular substance and abundant mature collagen fibrils with 64 nm periodicity. The fibrous cords extending from the nodules consisted of fibrocytes and varying numbers of myofibroblasts embedded in an abundant collagenous matrix; these cells and collagen fibrils were arranged in the same direction.

In the residual stage a scar-like tissue showing a few fibrocytes arranged sparsely within a collagenous matrix was found (Fig. 5). When compared with myofibroblasts, the fibrocytes had a relative narrow cytoplasm containing less abundant RER, less conspicuous Golgi complex, and no distinct microfilament bundles.

*Enzyme histochemistry.* In the nodules of both proliferative and involutinal stages, most cells probably representing myofibroblasts and fibroblasts exhibited strong activity of DAP IV (Fig. 6d). The cells in the residual lesions and in the fibrous cords of the involutinal lesions also displayed positive reaction, but the activity was weaker than that of the proliferative and the involutinal nodules.

Moderate to strong activity of ALP was noted in the perivascular mesenchymal cells of the proliferative and the involutinal nodules. The endothelial cells of small blood vessels also had high ALP activity, but the other cells were totally devoid of reaction products (Fig. 6c). While macrophages



**Fig. 6.** Enzyme histo section. **a** perivascular the right reveal active **b** Detail of ALP-positive activity with interspersed pinocytotic vesicles, cells and perivascular DAP IV in a frozen section are positive for DAP





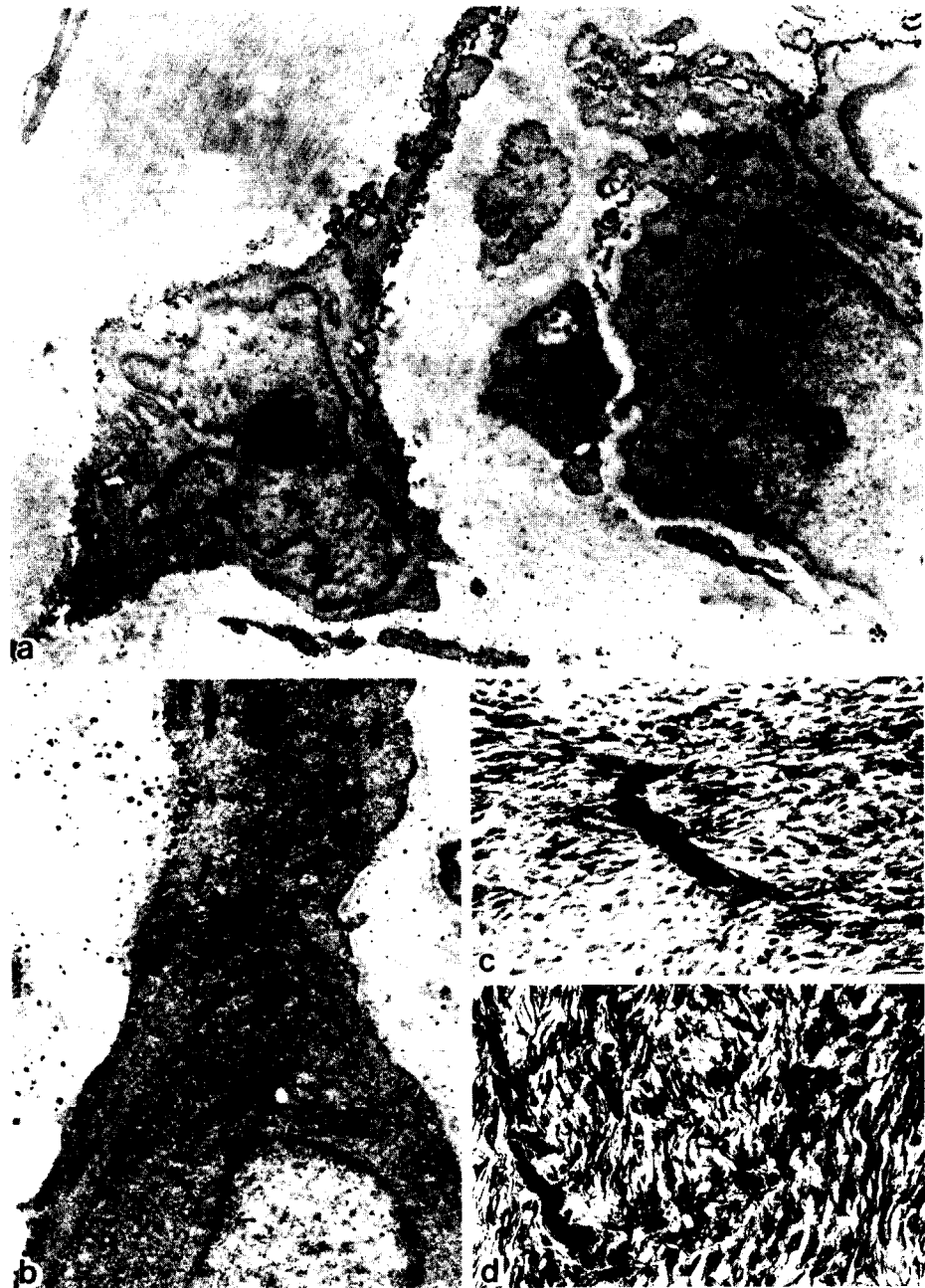
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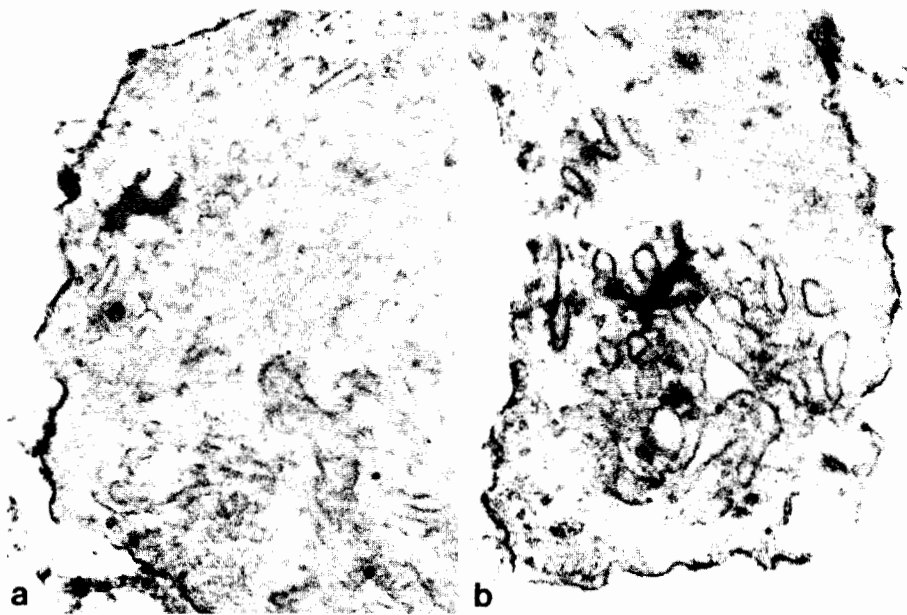
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**Fig. 6.** Enzyme histochemistry: **a** Electron microscopic demonstration of ALP in a vibratome section. A perivascular mesenchymal cell in the left as well as vascular endothelial cells in the right reveal activity of ALP located in the cell membrane and pinocytic vesicles.  $\times 9,900$ . **b** Detail of ALP-positive mesenchymal cell having well-developed RER and bundles of microfilament with interspersed dense patches (*arrows*). The reaction product is found chiefly in the pinocytotic vesicles.  $\times 17,600$ . **c** Distribution of ALP in a frozen section. Vascular endothelial cells and perivascular mesenchymal cells exhibit positive reaction.  $\times 243$ . **d** Distribution of DAP IV in a frozen section. Most of the proliferative cells and vascular endothelial cells are positive for DAP IV.  $\times 243$



**Fig. 7.** Electron microscopic immunohistochemistry for DAP IV. **a** Electron opaque reaction product is demonstrated on the cell membrane.  $\times 22,300$ . **b** Part of a DAP IV-positive cell having microfilament aggregates with several dense patches.  $\times 21,600$

displayed high activities of ACP and ANA-Est, the majority of cells constituting the lesions of each stage showed negative or very weak reaction for these enzymes.

*Ultrastructural enzyme histochemistry for ALP* demonstrated a strong reaction in the vascular endothelial cells and a moderate reaction in the perivascular mesenchymal cells (Fig. 6a). The reaction product was observed on the cell membrane, in the pinocytotic vesicles (Fig. 6b) and sometimes in the Golgi complex. These ALP-positive perivascular mesenchymal cells were interpreted as a variant of myofibroblasts, since they possessed microfilament bundles with dense patches (Fig. 6b), well developed RER, prominent Golgi complexes, indistinct basal lamina, and occasional intercellular junctions. These ALP-positive cells differed from pericytes, because they did not share a continuous basal lamina with endothelial cells. Furthermore, the distance between the ALP-positive myofibroblasts and the vessel wall was much greater than that between endothelial cells and pericytes.

*Ultrastructural immunohistochemistry for DAP IV.* By electron microscopy, DAP IV immunoreactivity was manifested as an electron opaque granular reaction product over the cell membrane of myofibroblasts (Figs. 7a, b), fibroblasts and fibrocytes as well as endothelial cells. There was some positive reaction in the Golgi complexes and RER, while mitochondria and lysosomes were not stained. No reaction was observed when the anti-DAP IV antibody was replaced with normal preimmune serum.

## Discussion

The results of our study are in agreement with those of Majnani and Iwasaki (1970) and Iwasaki et al. (1971) who demonstrated the involutonal changes in the nodules. Since the nodules are found in vivo as well as in vitro, it is surprising that many of the nodules are inactive.

Intracytoplasmic microfilaments are often in myofibroblasts and may be an important factor for the macroscopic size, the microfilament bundles seen in myofibroblasts (Iwasaki et al. 1971; Iwasaki et al. 1969); Iwasaki et al. (1971) presented abnormal relaxation cycle. The nodules relax into feltwork and contract; the nodules in the relaxed state aggregate in myofibroblasts and contract of myofibroblasts.

The enzyme histochemistry of palmar fibromatosis is similar to that of pericytes (Fukasawa et al. 1971). The nodules of the gastroduodenal tubular epithelium and the venules are composed of T lymphocytes and fibroblasts has no enzyme activity (Müller et al. 1971).

In the present study, the cell membrane of endothelial cells is stained as endothelial cells and enzyme possibly by an other possible amino acids (Gossrau 1981; Gossrau 1981).



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not stain. Furthermore,  
the vessel wall  
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Electron microscopy,  
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## Discussion

The results of our ultrastructural studies support the classic work by Gabiani and Majno (1972) who for the first time found myofibroblasts in palmar fibromatosis. As we have previously reported (Müller et al. 1983; Iwasaki et al. 1983a), most of the cells constituting the proliferative and the involucional nodules of palmar fibromatosis are myofibroblasts. Although Meister et al. (1979) stated that the majority of cells in the proliferative stage was compatible with fibroblasts, our ultrastructural studies demonstrated that immature fibroblasts were rare even in the early proliferative nodules. Since the recent studies indicated that myofibroblasts may proliferate in vivo as well as in vitro (Iwasaki et al. 1980 and 1983b), it is not surprising that myofibroblasts constitute the majority of cells in the proliferative nodules.

Intracytoplasmic aggregates of microfilaments were encountered most often in myofibroblasts of the involucional lesions. These structures may be an important evidence of cell contraction and are probably responsible for the macroscopic contraction observed clinically. Apart from their small size, the microfilament aggregates were reminiscent of cytoplasmic inclusions seen in myofibroblasts of infantile digital fibromatosis (Bhawan et al. 1979; Iwasaki et al. 1980). Using the heavy meromyosin binding method (Ishikawa et al. 1969); Iwasaki et al. (1983b) demonstrated that these inclusions represented abnormal contraction of actin filament bundles in the cytoplasm. Nagai et al. (1978) have found that microfilaments of the slime mold change their morphology corresponding exactly to the phase of the contraction-relaxation cycle. The cytoplasmic microfilament bundles transform themselves into feltwork ("skein body") in the shortening phase of maximal contraction; the microfilaments regain their parallel order and bundle structures in the relaxing phase. Therefore, it is possible that the microfilament aggregates in myofibroblasts of palmar fibromatosis similarly result from contraction of microfilament bundles.

The enzyme histochemical studies demonstrated that the cells composing palmar fibromatosis had a high activity of DAP IV. This enzyme is a membrane-bound peptidase in a variety of cell types (McDonald et al. 1971; Fukasawa et al. 1981; Gossrau 1981; Lojda 1981) including mucosal epithelium of the gastrointestinal tract (Gossrau 1981), salivary gland epithelium, renal tubular epithelium, liver cells (Fukasawa et al. 1981), endothelium of the venules and the venous part of capillary bed (Lojda 1979), and peripheral T lymphocytes (Feller et al. 1982), but the activity of DAP IV in myofibroblast has not been reported in the literature except in our recent work (Müller et al. 1983; Iwasaki et al. 1983a).

In the present study, DAP IV was found to be localized chiefly on the cell membrane of myofibroblasts, fibroblasts, and fibrocytes as well as endothelial cells. The physiological role of DAP IV is obscure, but the enzyme possibly cleaves some peptide substances produced by the cells; an other possibility is a role in the transport (secretion and reabsorption) of amino acids and peptides across the cell membrane (Fukasawa et al. 1981; Gossrau 1981). Therefore, DAP IV in the connective tissue cells may

play a certain role in metabolism of intercellular matrix by regulating breakdown or secretion and reabsorption of polypeptides of tropocollagen and other ground substances. The enzyme also may be used as a functional marker of the fibroblast-myofibroblast group of the mesenchymal cells.

Significance of the ALP activity in the perivascular mesenchymal cells is not clear. Since these cells otherwise have the typical morphology of myofibroblasts, they are interpreted as a type of this cell. Katenkamp and Stiller (1976) reported that pericyte-like cells somewhat distant from blood vessels were present in palmar fibromatosis. These pericyte-like cells are similar to the perivascular myofibroblast in the present study, except that the pericyte-like cells lacked well-developed RER. Although the relation between pericyte and myofibroblast has not been established, the perivascular myofibroblasts may represent the transitional form from the pericyte to the ordinary myofibroblast.

The a etiology of palmar fibromatosis still remains obscure. However, there is little doubt as to the existence of a hereditary disposition. Ling (1963) suggested that a single gene, behaving as a Mendelian dominant, was likely to be involved. Although several investigators mentioned that palmar fibromatosis was more common in patients having diabetes mellitus (Weckesser 1964), chronic alcoholism, chronic liver disease or epilepsy (Pojer et al. 1972), the exact significance of these coexisting diseases was unknown.

The other causative factor may be minor trauma producing partial rupture of palmar fascia, followed by reparative fibroblastic proliferation (Larsen et al. 1960). In the present study, the proliferative nodules of palmar fibromatosis often contained small foci of perivascular haemorrhage and haemosiderin deposition accompanied by accumulation of macrophages and some lymphocytes. These macrophages and/or lymphocytes possibly secrete a growth factor that induces proliferation of hereditarily abnormal fibroblasts and myofibroblasts giving rise to palmar fibromatosis.

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