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Department of Morphology, Department of Hand Surgery, Department of Clinical Laboratory, National Institute of Traumatology, Budapest,
Department of Traumatology, Markusovsky Hospital, Szombathely, Hungary

Fine structural alterations of the palmar aponeurosis in Dupuytren's contracture

A combined scanning and transmission electronmicroscopic examination

By **L. Józsa, A. Salamon, A. Réffy, A. Renner, S. Demel, A. Donhöffer, T. Pintér, J. Thöring**

Ultrastrukturveränderungen der Palmaraponeurose bei Dupuytren-Kontraktur

Eine kombinierte Untersuchung mit dem Raster- und Transmissionselektronenmikroskop

Zusammenfassung

Chirurgisch entnommenes Gewebe von 29 Männern und 9 Frauen mit Dupuytren-Kontraktur und Anteile der Palmaraponeurose von 9 durch Unfall verstorbenen Personen ohne Hinweise auf Dupuytren-Kontraktur wurden im Transmissions- und/oder Scanning-Electronenmikroskop untersucht. Bei Dupuytren-Kontraktur wurden klassische Fibroblasten und Myofibroblasten gefunden. Letztere bildeten etwa ein Viertel der nicht-entzündlichen Zellen. Bei der Dupuytren-Kontraktur war die Orientierung der dünnen und der dicken Kollagenfasern unregelmäßig und variabel. Die Paccini-Körper erwiesen sich als hypertrophiert. In der Umgebung bestand eine deutliche Zunahme des kompakten kollagenen Bindegewebes.

Sachwörter: Dupuytren-Kontraktur, Palmaraponeurose, Fibromatose, Myofibroblast, Elektronenmikroskopie

Abstract

The authors examined the fine structural alterations of palmar aponeurosis in Dupuytren's contracture. Two types of the fibroblasts can be identified: classic fibroblasts and myofibroblasts. In the Dupuytren's tissue one fourth of all non-inflammatory cells were regarded as myofibroblasts. In the Dupuytren's aponeurosis the orientation of the thin and thick collagen fibers varied. The Paccini corpuscles were hypertrophised and around them compact collagen tissue was increased.

Dupuytren contracture is characterized by nodular proliferation of the palmar fascia which leads to flexion contracture of the fingers. The exact mechanism by which contracture occurs in Dupuytren's disease is not clear. Gabbiani and Majno (3) discovered the existence of contractile myofibroblasts and the disease is an active cellular process. In Dupuytren's contracture the myofibroblasts are present only in the nodules (4) and are regarded responsible for the deformity of the fingers (1, 4, 6, 16, 17, 19, 20). Dupuytren's disease can lead to the appearence of abnormal collagen fibrils (2, 10, 14, 15). Also specific biochemical alterations are detected in Dupuytren's contracture, for example, increase of total glycosaminoglycan content, increase of total collagen content, higher lysil oxidase activity etc. in the palmar aponeurosis (6, 7, 9). Since Dupuytren's report there have been numerous studies on the pathogenesis of the disease, but there is still controversy as to whether the main pathological changes are concerned with cellular or extracellular components of the palmar aponeurosis.

To our knowledge, an examination of the palmar aponeurosis with a combination of transmission and scanning electronmicroscopy has not yet been carried out. The purpose of this study was to investigate the ultrastructural and stereological alterations in the palmar aponeurosis in Dupuytren's disease.

Material and methods

The palmar aponeurosis was examined in 12 patients with both scanning and transmission electron microscopy, and in 26 patients with the scanning electron microscope alone. The specimens were obtained during surgery from 29 male and 9 female patients, 32 to 56 years of age. Normal palmar aponeuroses were taken from 9 healthy subjects within 2 h after an accident-induced death.

For transmission electron microscopy the tissue pieces were fixed in 2% osmium tetroxide buffered after Millonig at *p*H 7.4, then dehydrated in grading alcohols and embedded in DURCUPAN ACM. The semithin and ultrathin sections were cut with a Reichert-Jung Ultracut ultramicrotome. The semithin sections were stained with toluidine blue and the ultrathin sections with uranile acetate and lead citrate. The ultrathin sections were examined with TESLA 500 BS electron microscope.

For scanning electron microscopy the tissue samples were fixed in solution of 2% glutaraldehyde buffered with cacodylate at *p*H 7.4. Thereafter they were dehydrated in grading alcohols and dried in a critical point dryer apparatus (Balzer's). Other tissue samples (after fixation) were frozen in liquid nitrogen at —160°C, and freeze-fractured.

The specimens were coated with gold and examined in a TESLA BS 300 scanning electron microscope.

Using a routine light microscope the total number of non inflammatory cells were counted from semithin sections and the number of myofibroblasts were counted from ultrathin sections corresponding the semithin sections (12).

Results

1. Transmission electron microscopy: In the extracellular space there are regions of compact collagen tissue, often with microfibrils. In any spaces loose collagen tissue can be identified with an increased amount of fibrils regarded as either type III or type I collagen. The thick collagen fibrils (probably type I fibrils) seem to be regular in shape and variable in diameter (Fig. 1). Around the myofibroblastic cells a fine granular ground substance is observed. In palmar aponeurosis the main cell type found is the fibroblast-like cell. Based on the morphological features of the fibroblasts, increases in two cell types can be identified:

a) Classic fibroblasts, which have irregular cell contours with elongated cytoplasmic pseudopodia. These cells contain a few mitochondria and lysosomes. They have a well developed smooth and/or rough endoplasmic reticulum, its cysternae are distended and contain fine granular material.

b) Myofibroblasts which contain a well developed system of myofilaments. The filaments are 4 to 6 nm in diameter and these filaments are situated beneath the plasma membrane in a parallel fashion. The organelle content of these cells varies, but there are always some mitochondria, rough endoplasmic reticulum and free ribosomes (Fig. 2).

In addition to the two types of fibroblasts mentioned above there are inflammatory cells in the altered aponeurosis. In the studied tissue samples one fourth of all non-inflammatory cells were regarded as myofibroblasts (Table 1).

Table 1. The percentage of inflammatory, cells fibroblasts and myofibroblasts in the studied aponeuroses

	Inflammatory cells %	Fibroblasts and Fibrocytes %	Myofibroblasts % of all non-inflammatory cells
Normal aponeurosis (n = 9)	8 (0—13)	92 (87—100)	2 (0—5)
Dupuytren's aponeurosis (n = 12)	26 (20—31)	74 (69—80)	23 (16—30)

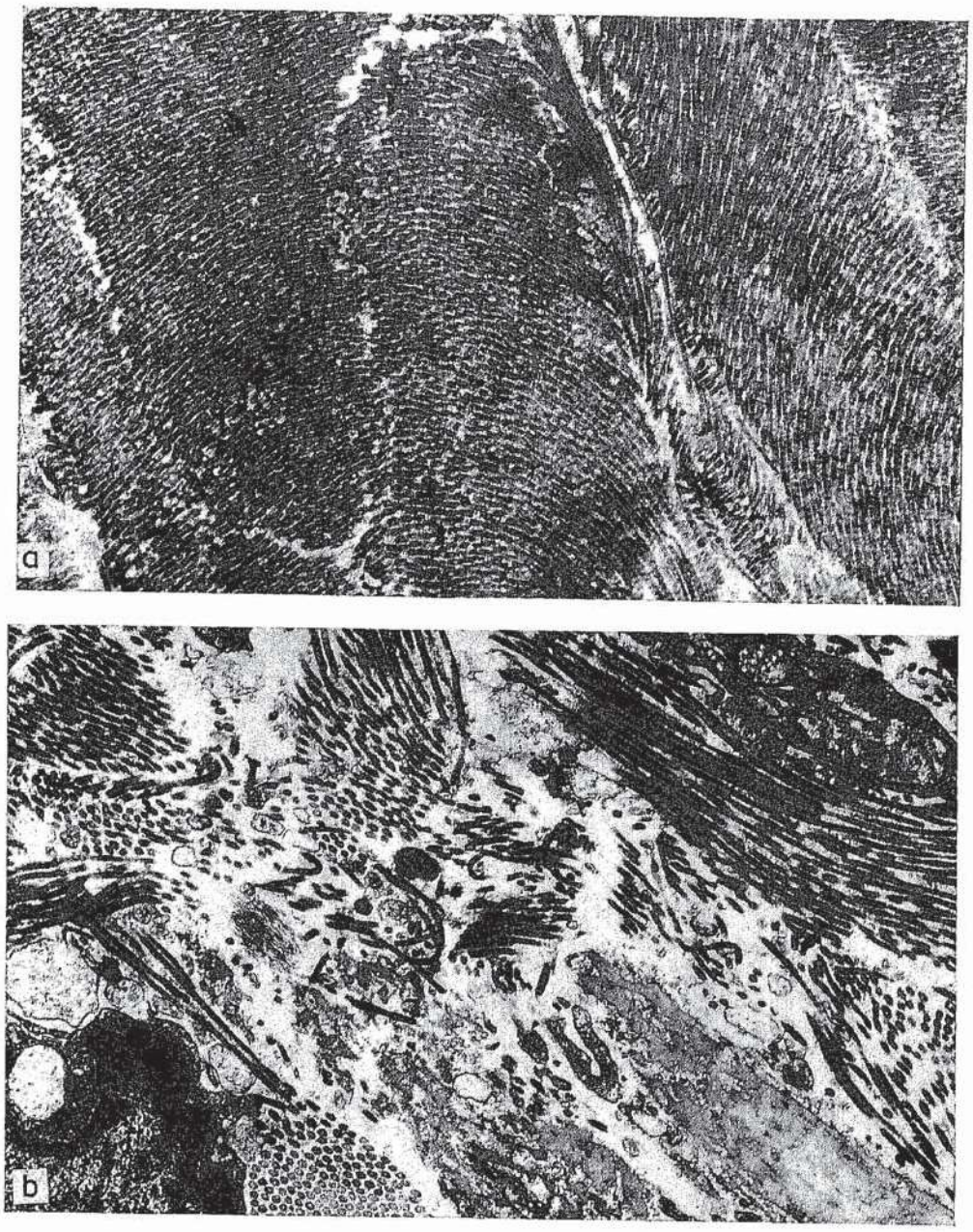
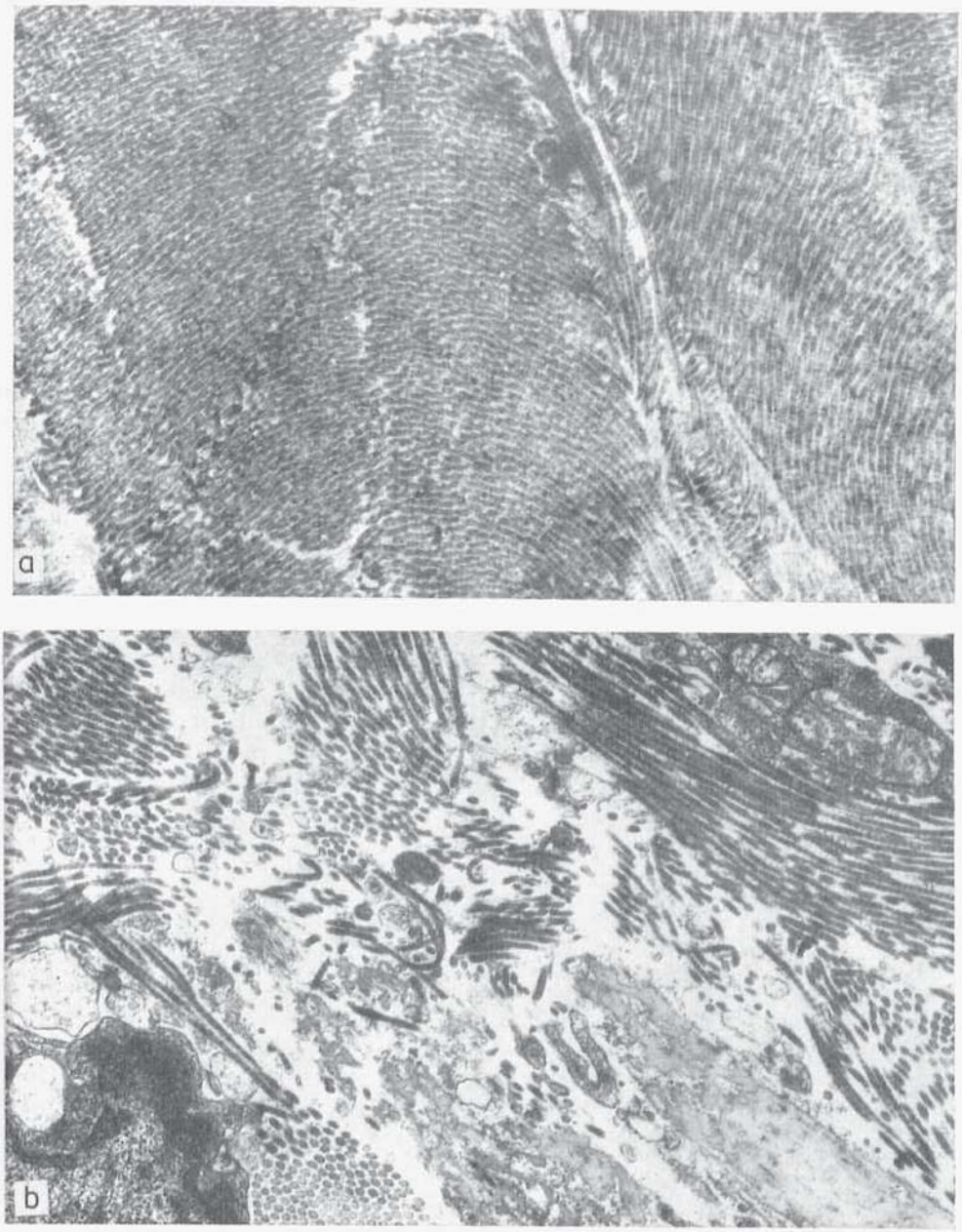


Fig. 1.a) Collagen structure of the normal aponeurosis. ×17220. b) In spaces, loose collagen tissue can be identified with microfibrils in the Dupuytren's aponeurosis. ×17220.



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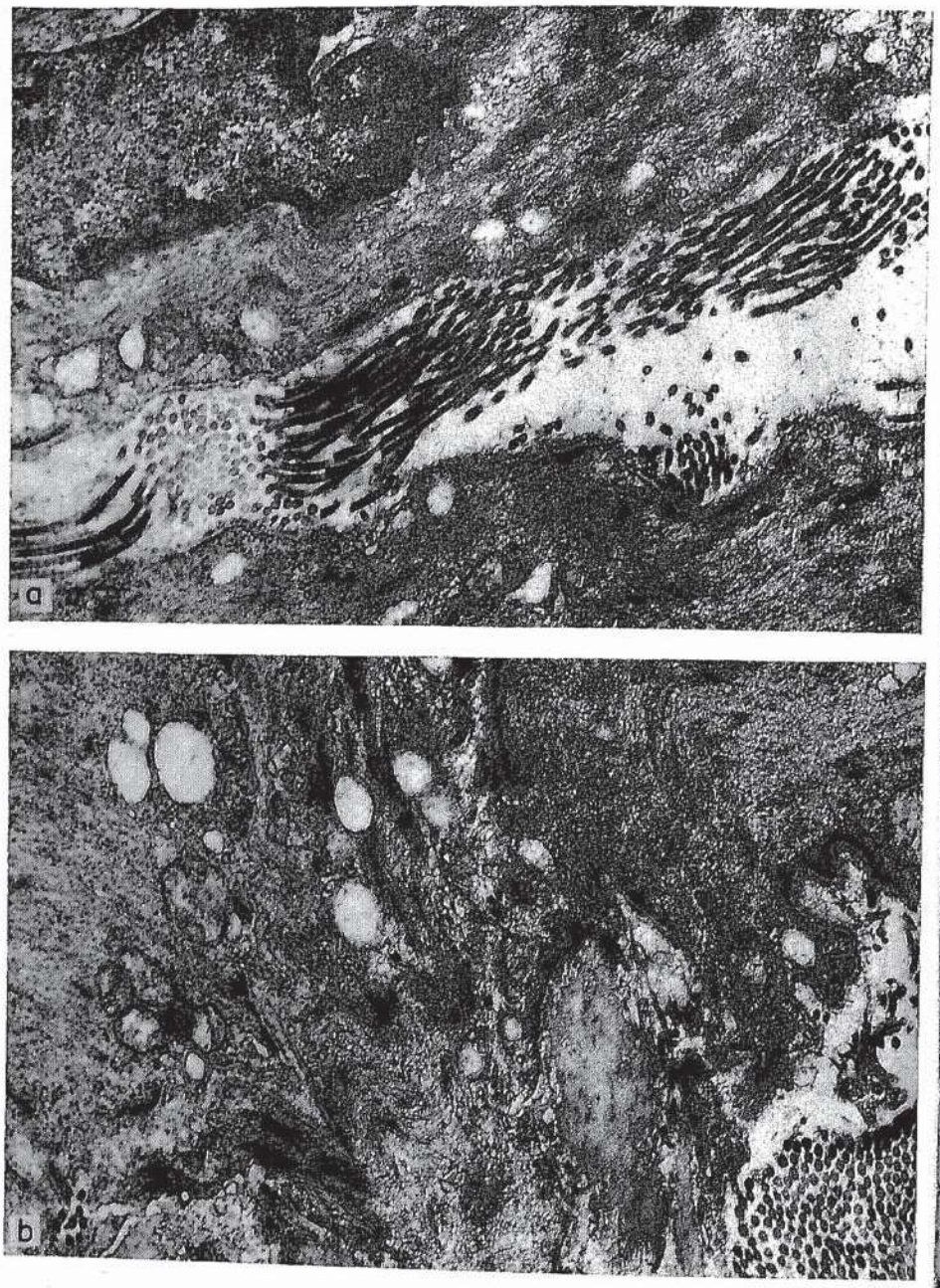
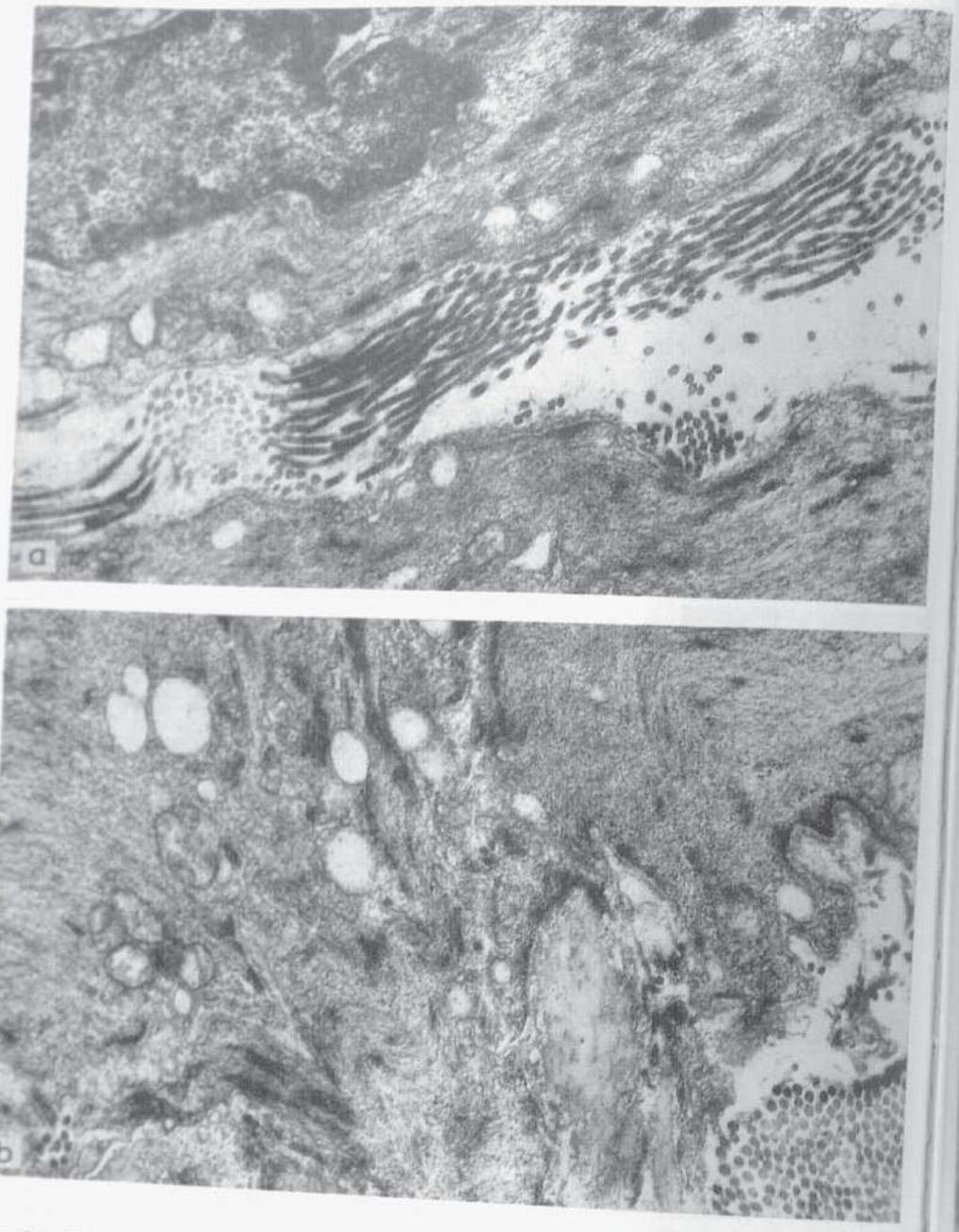


Fig. 2.a) Myofibroblasts in the Dupuytren's nodules. ×20500. b) Well developed system of myofilaments in the myofibroblastic cell. ×20500.



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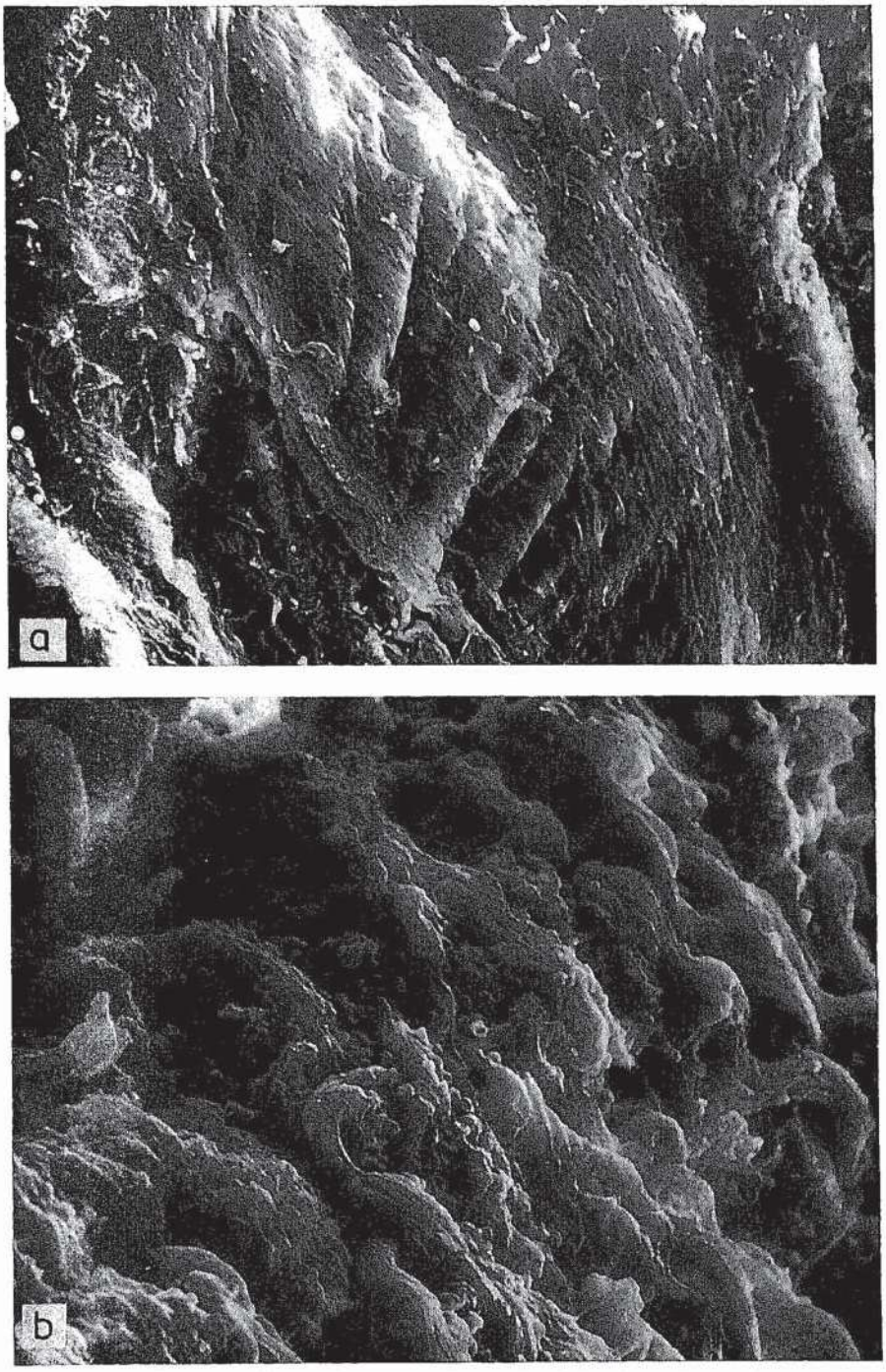
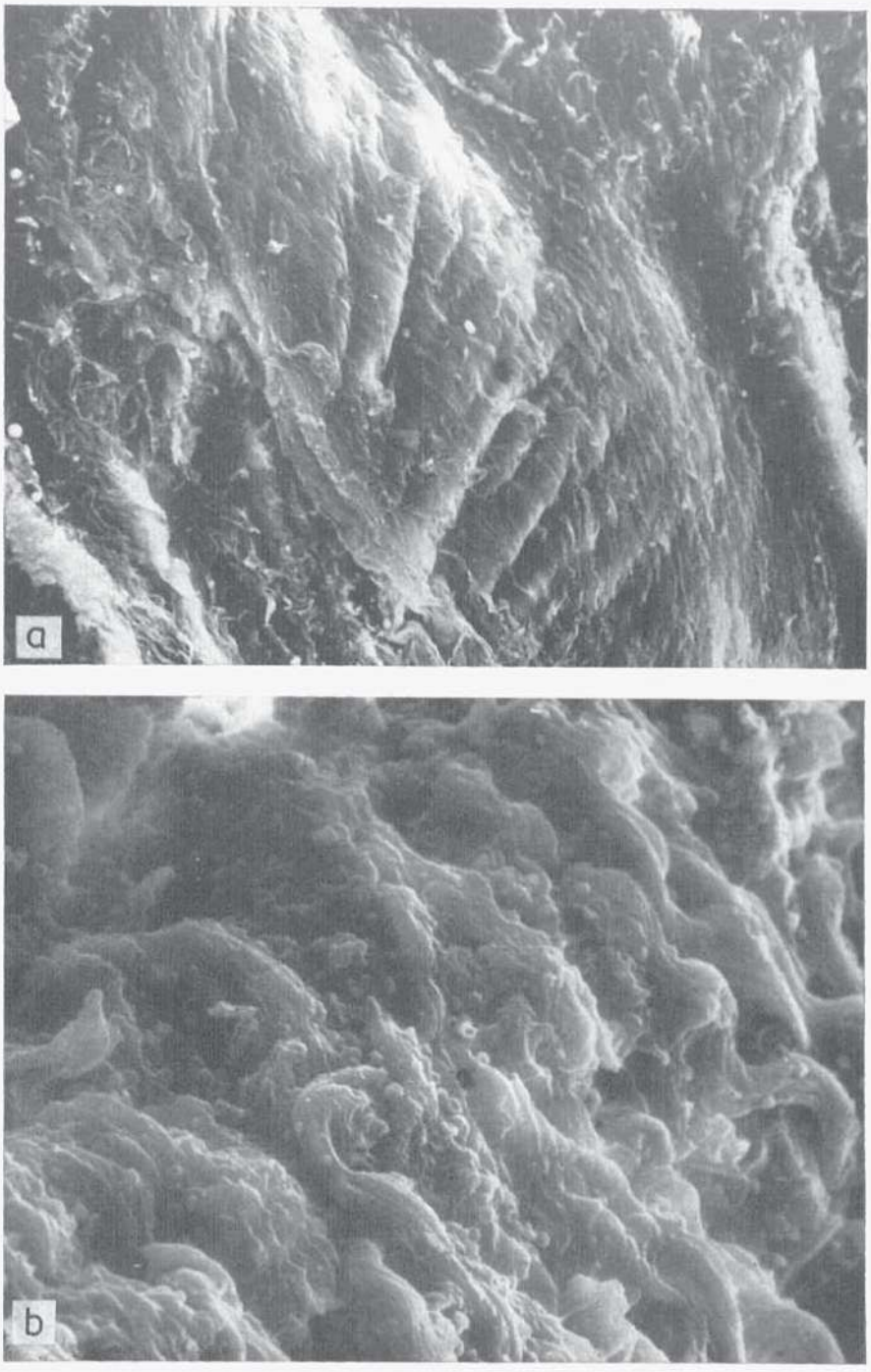


Fig. 3.a) Surface of the normal palmar aponeurosis. ×3000. b) Internal structure of the freeze fractured distal part of normal aponeurosis. ×3200.



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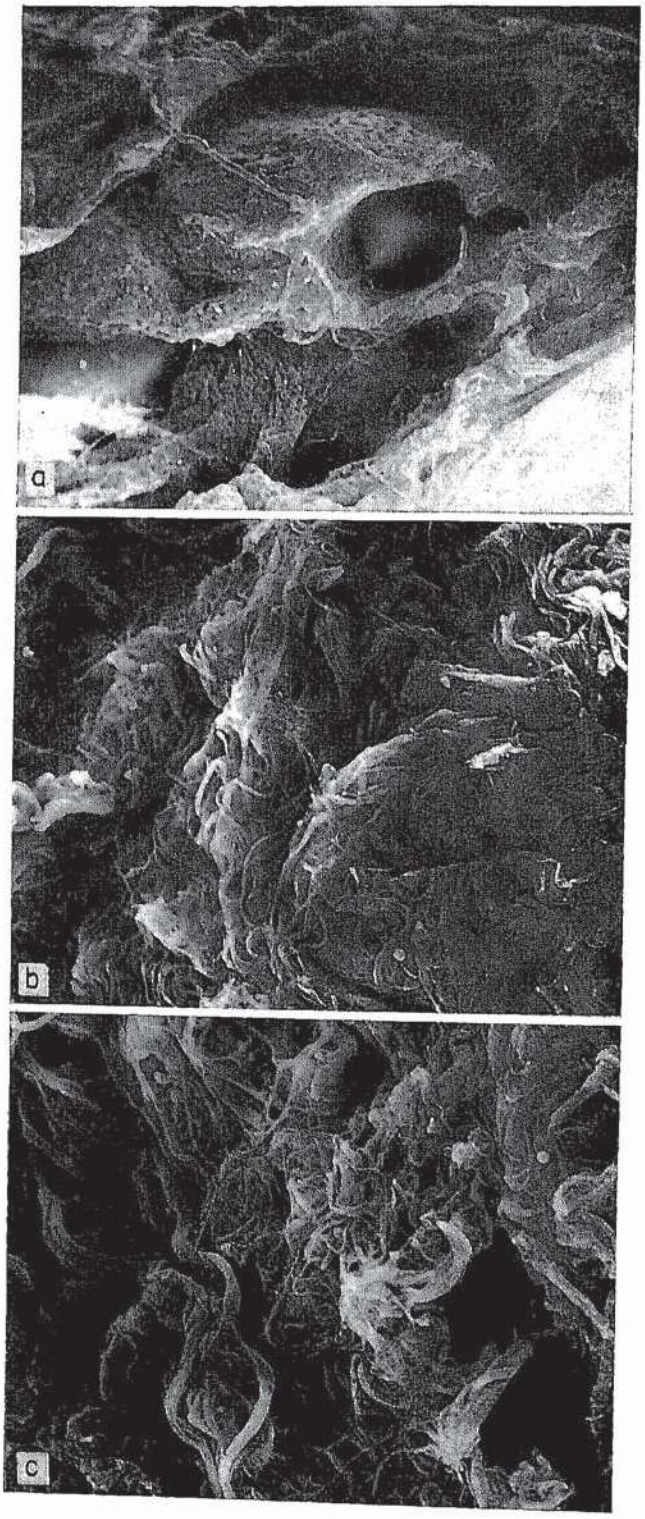
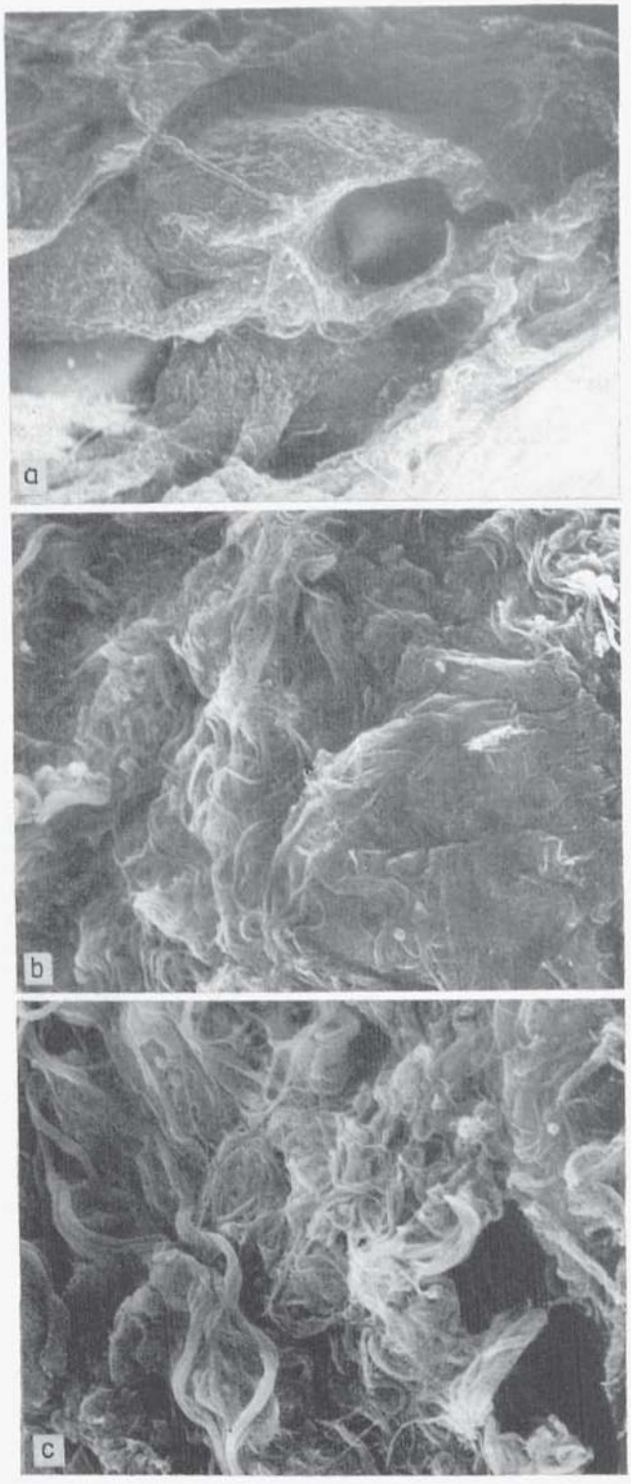


Fig. 4.a) Dupuytren's nodule. ×2300. b) Surface of the Dupuytren's nodule. ×4180. c) Internal structure of the Dupuytren's nodule. ×5320.



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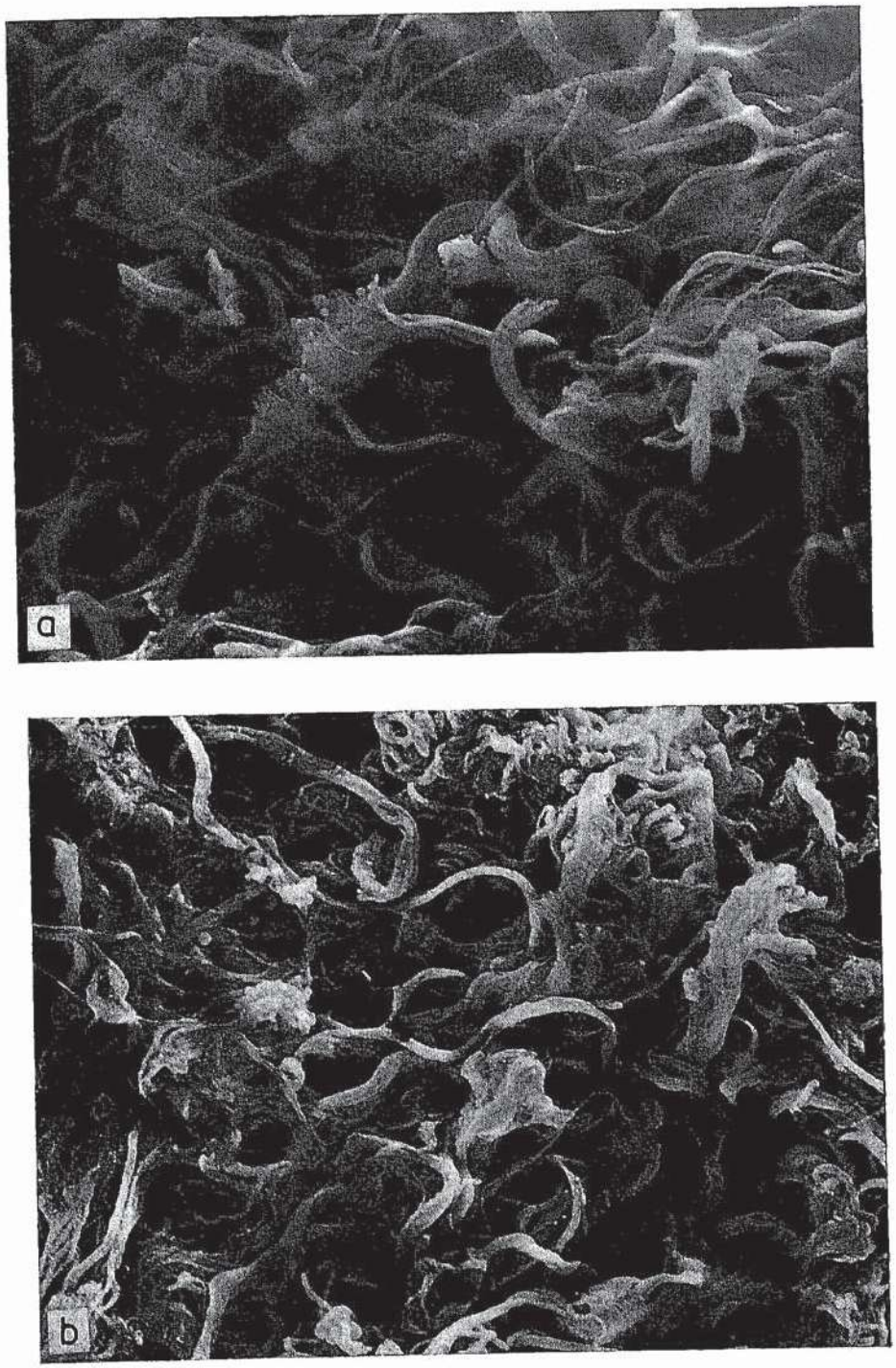
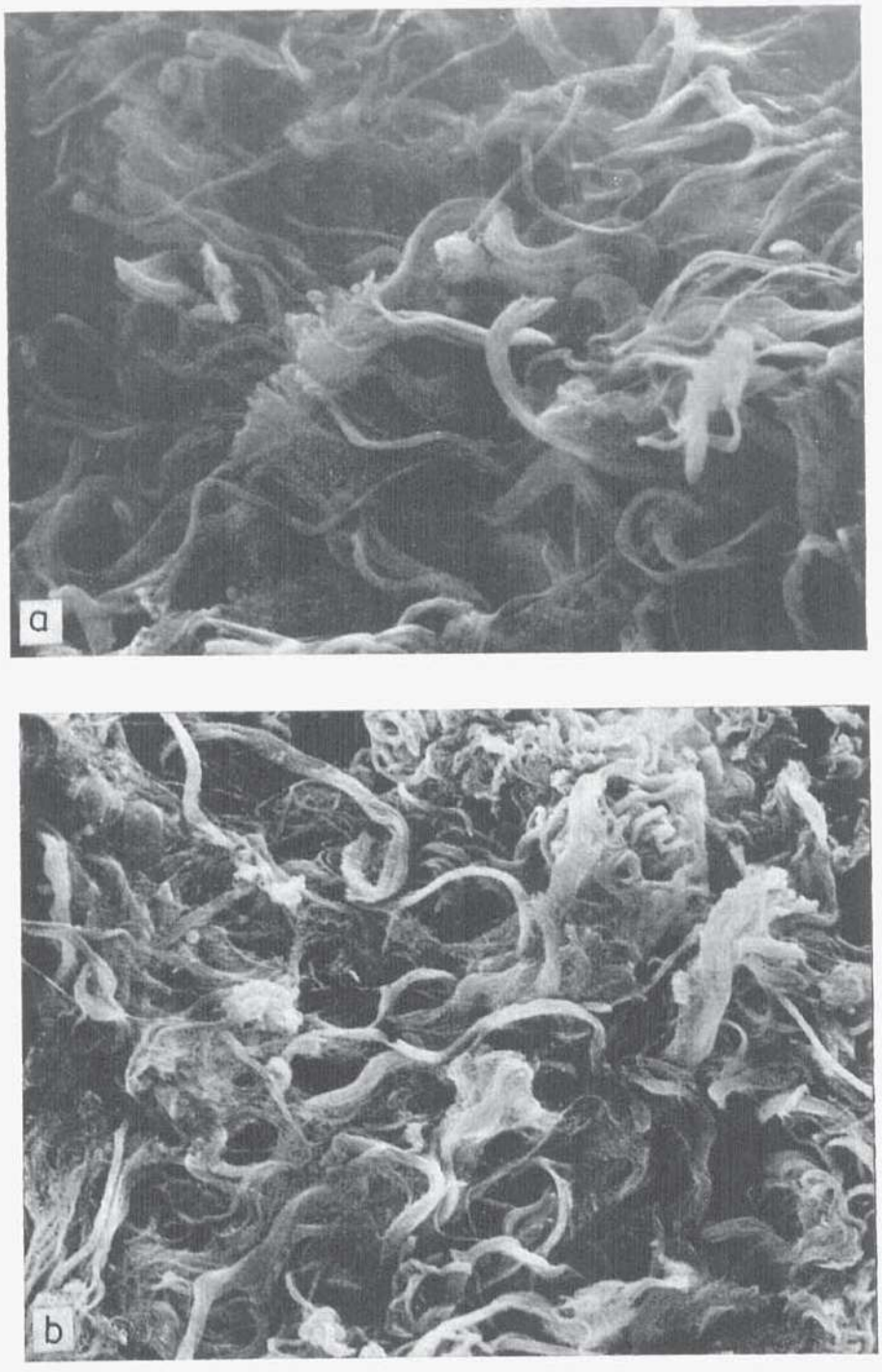


Fig. 5.a) Cord without nodule in Dupuytren's contracture. ×6000. b) Fiber composition and orientation very similar as in nodules. ×6000.



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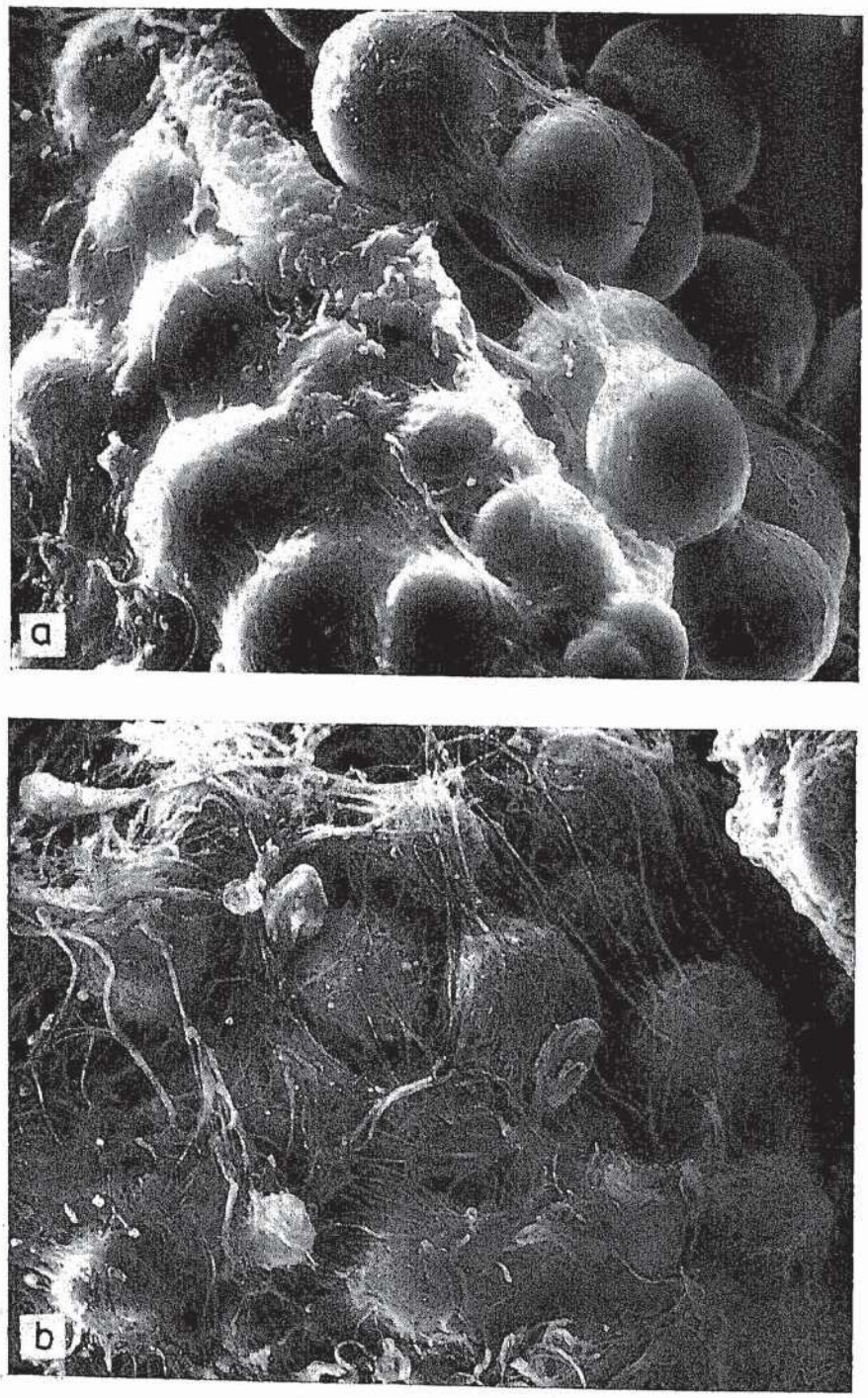
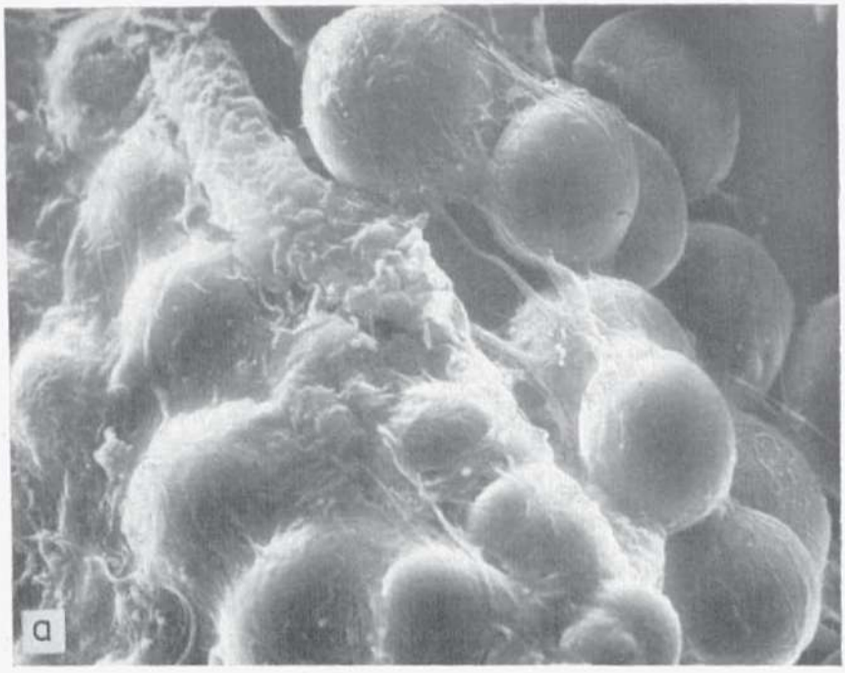
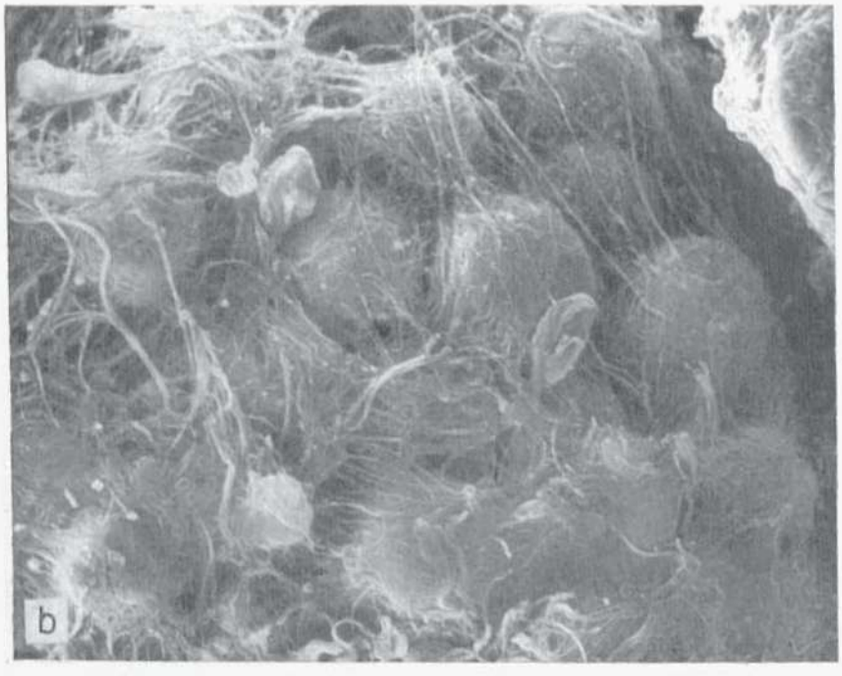


Fig. 6.a) Lipid cells in the normal palmar aponeurosis. ×3000. b) The intraaponeurotical fat tissue is atrophied by the proliferated connective tissue. ×3000.





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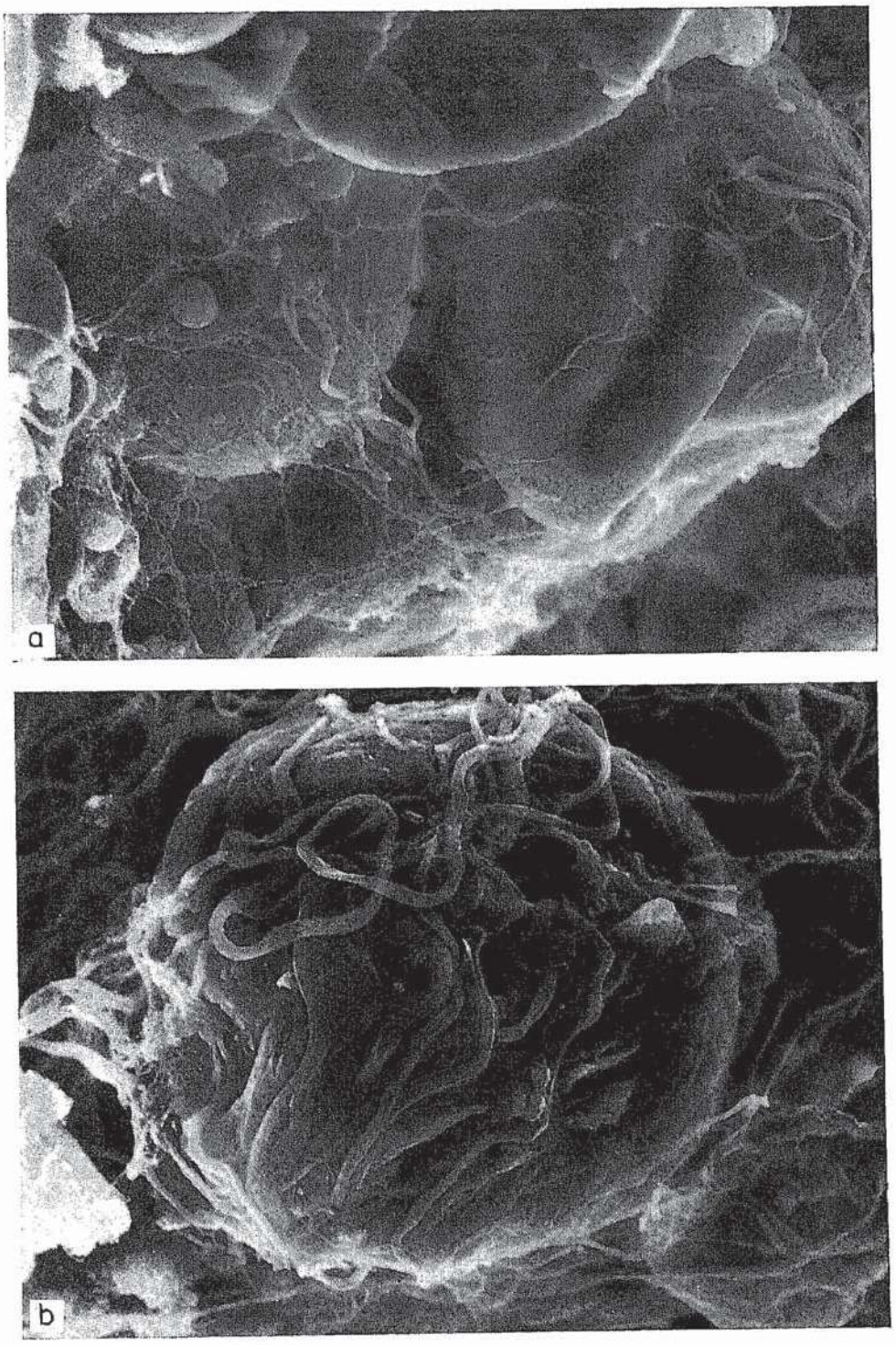
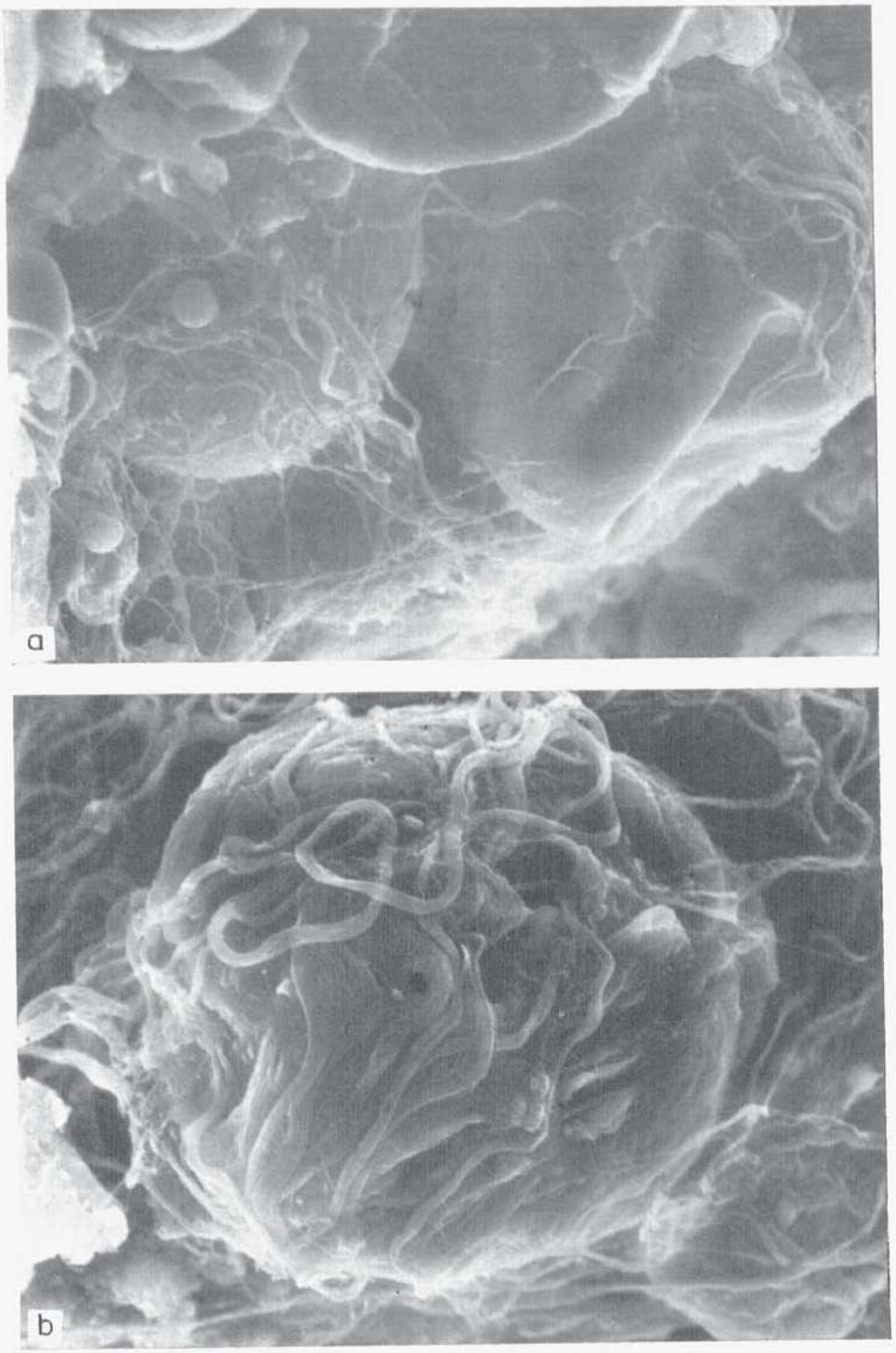


Fig. 7.a) and b) The Paccini corpuscles are hypertrophied and around them increased compact collagen tissue can be seen. ×13600.



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2. Scanning electron microscopy: The proximal region of the normal aponeurosis consisted of well organized, longitudinally oriented, thick (type I) collagen fibers. On the distal region of the normal aponeurosis longitudinally, transversally and vertically oriented fibers (Fig. 3) can be distinguished clearly. In Dupuytren's nodule the orientations of the fibers was varied; in some regions a fiber network could be seen: The orientation both of the thin and thick fibers varied (Fig. 4). Usually there were areas of disorganized collagen. The amount of thin fibers (probably type III collagen) was 40—60 per cent. In some regions play formation could be detected between the thin and thick fibers. In the cord (without nodule) of Dupuytren's disease the fiber composition was very similar to that in nodules (Fig. 5). In the cords too thin collagen fibers were 40—60% of all inflammatory cells. The collagen fibers which were perpendicular to the skin showed massive cross-linking and thickening. The intraaponeurotical fat tissue is atrophied by the proliferated and thickened connective tissue (Fig. 6). The Paccini corpuscles are hypertrophized and around them compact collagen tissue can be identified with an increased amount of thin fibrils (Fig. 7).

Discussion

Gabbiani and Majno (3) found that the fibroblasts had changed into contractile fibroblasts and that the consequent contraction played a major part in the pathogenesis of contracture. The presence of myofibroblasts is characteristic of Dupuytren's disease. On the other hand specific biochemical changes were found in diseased aponeurosis. The major biochemical changes in Dupuytren's contracture include increased amounts of type III collagen (4, 7, 13, 17) and the hexosamine, glycosaminoglycane (5). Fibronectin (11) content is also increased in affected aponeurosis. Increased amount of hydroxilysine, hydroxyproline (6) and an increased number of reducible cross-links have been found in Dupuytren's fascia. Increased synthesis of type III collagen has been shown to be related to the presence of myofibroblasts (1, 4, 8, 13).

We believe that the myofibroblasts have a central role in the clinical symptoms of contracture but the pathogenetic significance of myofibroblasts is not clear. In Dupuytren's contracture they are present only in the nodules and are regarded as responsible for the deformity of the fingers. The formation of type III collagen must be an integral part of the disease process because type III collagen was always found both in the nodule and in the cords. We mentioned that the loss of the orientation of the collagen fibers also has a pathogenetic role in the appearence of the contracture. The pathologically altered aponeurosis shows a variable morphology, increase of myofibroblasts and the collagen fibers display major variations both in diameter and orientation. The role of alterations of the Paccini corpuscles is questionable.

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Correspondence to Dr. Dr. sc. med. L. Józsa, Országos Traumatol. Intézet, Morphologiai Osztály, Mezó Imre ut 17, PF 21, H - 1430 Budapest, Ungarn.

Hämatologie: ZFA-Taschenatlas/Patricia E. Hewitt. — Stuttgart: Hippokrates Verlag, 1986. — VI, 82 S.: 141 farb. Abb. ISBN 3-7773-0795-5 geb.: DM 24,80.

Mit der Herausgabe der Reihe ZFA-Taschenatlanten, die der Zeitschrift für Allgemeinmedizin zugeordnet sind, wird beabsichtigt, allgemeinmedizinischen Ärzten, aber auch Klinikern und Studenten optische Informationen über häufige Krankheitsbilder zu vermitteln. Dieses Ziel wird auch mit dem vorliegenden Titel verfolgt. Grundkenntnisse werden vorausgesetzt. Darüber hinaus möchten die Autoren zum gegenseitigen Verständnis von Klinik und Laboratorium beitragen. Die Basis bilden eindrucksvolle Patientenfotos. In typischen Fällen sind auch charakteristische Blutbildveränderungen abgebildet und Röntgenaufnahmen beigegeben. Die Qualität der farbigen Abbildungen ist gut.

Es werden Erkrankungen mit Erythro- und Leukozytenveränderungen wie Anämien und Hämoglobinopathien, akute Leukämien, myeloproliferative Erkrankungen und chronisch lymphoproliferative Erkrankungen dargestellt. Weiterhin findet man Abbildungen zur hämorrhagischen Diathese, zu Koagulopathien sowie zu infektiöser Mononukleose und Malaria. Die Abbildungen werden durch kurze Texte ergänzt, dabei war nicht beabsichtigt, einen hämatologischen Atlas oder ein Lehrbuch zu ersetzen. Problematisch erscheint aus der Sicht der allgemeinen Pathologie die Verwendung des Begriffes „Zellulitis". Den Abschluß bildet ein Sachregister. D. Stiller (Halle)

Pathology in surgical practice/ed. by G. J. Hadfield; M. Hobsley; B. C. Morson. — London: Arnold, 1985. — XII, 500 S.: Abb., Tab. ISBN 0-7131-4471-8 cloth: £ 49.50.

Der Titel macht neugierig, ob es ein Buch mehr für Chirurgen oder mehr für Pathologen ist. Aus dem Vorwort erfährt man die Absicht, aus beiden Disziplinen und für beide zu schreiben, zur Förderung des gemeinsamen Herangehens bei der medizinischen Betreuung einschließlich der prognostischen Aussage für den einzelnen Patienten. Das Vorhaben ist gelungen. Es ist ein Werk entstanden, dessen einzelne Kapitel Autoren aus beiden Fachgebieten haben und klinische, diagnostische und therapeutische Aspekte beinhalten. Nach einer Einleitung der Herausgeber zum Thema „Der Chirurg und der Pathologe", die von der Ausfüllung von Vordrucken bis zum Wert eines pathoanatomischen Museums reicht, folgen die umfangsmäßig unterschiedlichen Organ- bzw. Fachgebiets- oder Sachkapitel in dieser Reihenfolge: Mamma, Verdauungstrakt, Lymphoretikuläres Sy-