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ORIGINAL COMMUNICATIONS

The pathogenesis of Dupuytren's contracture: Contractile mechanisms of the myofibroblasts

The role of myofibroblasts in the pathogenesis of Dupuytren's contracture was investigated by light and electron microscopic histochemical methods. Dupuytren's myofibroblasts contain an intracellular contractile mechanism that is driven by the dephosphorylation of adenosine triphosphate. Our study of calcium adenosinetriphosphatase (ATPase) activities verifies that the site of this energy system is on the myofilaments of the myofibroblasts. The degree of ATPase activity, as determined by cell counts, appeared to correlate with the residual contracture as predicted by the Legge and McFarlane Outcome Standard Formula. Further, alcian blue staining on the ultrastructural level indicates that the myofibroblasts are associated with each other and with surrounding collagen by a glycosaminoglycan matrix 300 to 1000 Å thick. Collagen fibrils are attached by a similar matrix comprised of 100 Å thick fibrils. The dynamic cellular architecture of the multiple adjacent myofibroblasts with their connections to surrounding collagen may be partially responsible for the residual clinical deformities seen in this disease. (J HAND SURG 8:235-243, 1983.)

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The mechanism by which joint contractures occur in Dupuytren's disease is not yet known. Distinctive nodules and contracted longitudinal bands of the palmar fascia are recognized as the clinical criteria for this condition. As early as 1959, Luck[1] stated that the joint contractures occur due to nodular contraction. He described the involutional stage as ending with the disappearance of these nodules and at this point contractures remain unchanged. The nodules are highly cellular and composed of myofibroblasts first described in the early 1970s by Gabbiani and Majno.[2] These myofibroblasts were found to have many of the ultrastructural properties of smooth muscle.[3-8] There have been many investigations dealing with the collagen biochemistry in Dupuytren's disease. It is now well established that considerable amounts of type III collagen are present in the palmar fascia of these patients.[4, 9-15] In Dupuytren's disease there are increased proportions of glycosaminoglycans in the palmar fascia, but the ultrastructural architecture has not been stressed.[5, 7, 10, 15-17] In previous investigations, a physical arrangement of the glycosaminoglycans to the myofibroblasts has not been formulated. It is generally agreed that myofibroblasts are contractile cells; however, the energy mechanism of the actomyosin filaments has not been previously defined in Dupuytren's tissue.

We therefore undertook a histochemical investigation on the light and electron microscopic levels to expand upon the cellular and molecular architecture of

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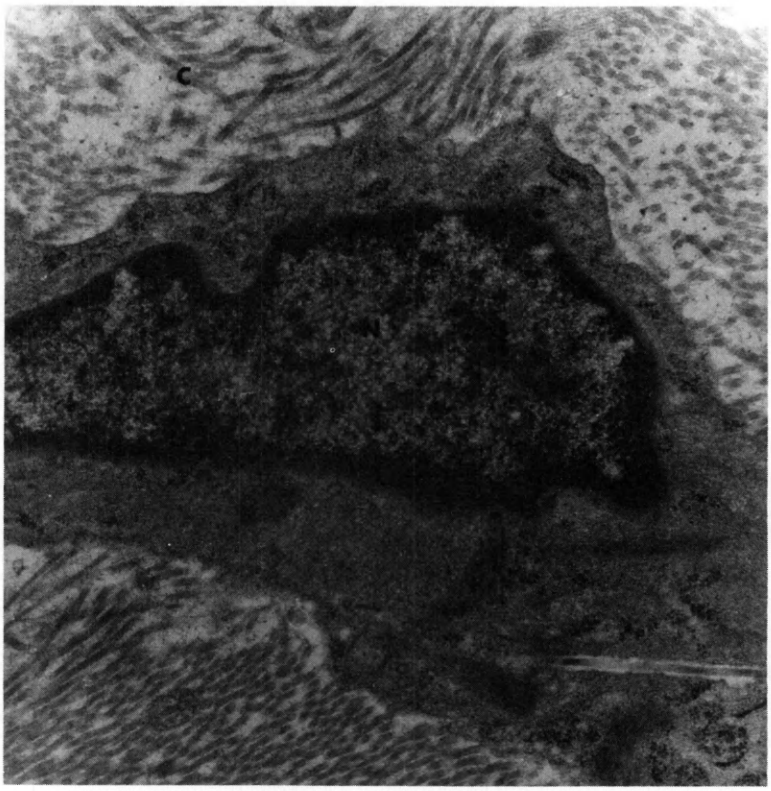

Fig. 1. Electron micrograph (×20,000) of a nodular myofibroblast. A prominently indented nucleus *(N)* and myofilament *(F)* bundles are evident. Collagen fibrils *(C)* border the cell.

the myofibroblast-collagen complex. In addition, we sought to define the energy mechanisms necessary for contraction of the actomyosin filaments. A correlation between the clinical signs[14] and the cellular biology has been suggested in the past, but a relationship between the myofibroblasts and the postsurgical residual contracture has not been previously made.

Materials

Tissue was obtained from 20 patients (21 cases, 19 men and two women) with Dupuytren's contracture of the hand. The dominant hand was involved in 17 patients. The average age was 59 years, the range being 36 to 73 years. Partial fasciotomies were performed in all cases. Control samples of palmar fascia were obtained from six patients undergoing hand surgery for other reasons.

Methods

For light microscopy, specimens from the nodular area and the proximal fascial cord were frozen in liquid nitrogen, cryoprotected in isopentane, and subsequently sectioned at 12 μ in a microtome/cryostat at −20° C. Sections were stained for calcium adenosinetriphosphatase (ATPase) activity in a buffered adenosine triphosphate (ATP) medium[18] after preincubations in Tris at pH 4.3, 7.2, and 10.4. Sections were also stained with alcian blue at pH 6.5 and hematoxylin.

For electron microscopy, 1 mm^3 samples of nodular areas and proximal cord were fixed either in 3% glutaraldehyde or in 3% glutaraldehyde containing 1% alcian blue at pH 6.5.[19] Cryostat sections were incubated in Tris hydrochloride buffer medium containing 50 mg/6 ml ATP at 37° C for 1 hour to demonstrate ATPase activity at pHs 4.3, 7.2, and 10.4. Control sections were incubated in a similar medium without ATP. Subsequently, all samples were washed in buffer, postfixed in 1% osmium tetroxide, dehydrated in an increasing ethanol series, cleared in propylene oxide, and embedded in Polybed 812 (Polysciences, Inc., Warrington, Pa.). Sections were cut on an ultramicrotome at 500 Å, stained in uranyl acetate, poststained in lead citrate, and photographed with an HU-12 electron microscope.

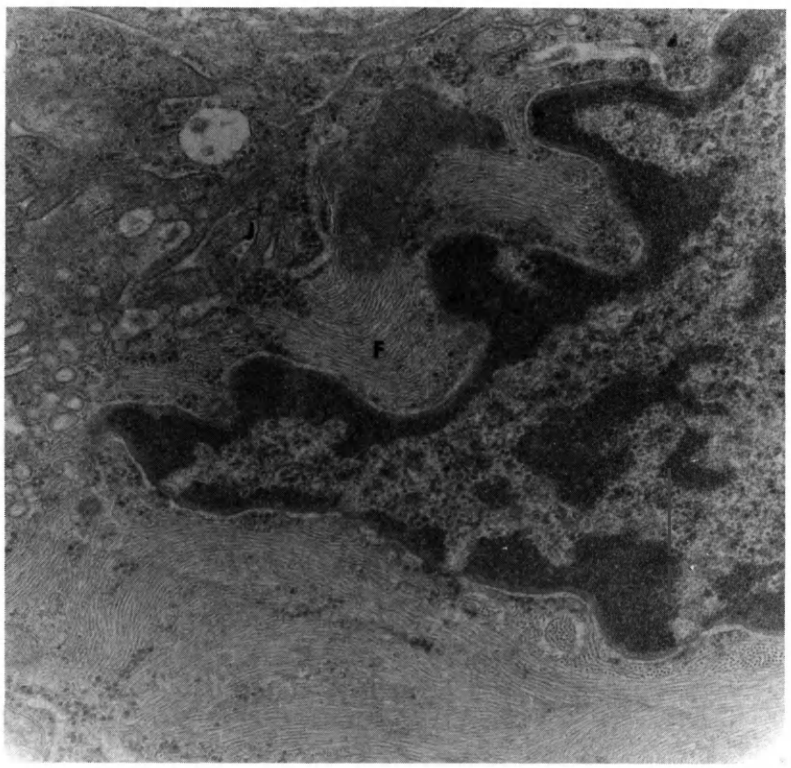

Fig. 2. Electron micrograph (×30,000) of a nodular myofibroblast. Myofilaments *(F)* fill the cell cytoplasm. Junction membrane complexes *(J)* are evident.

Results

Nodule of Dupuytren's disease. Myofibroblasts (Figs. 1 and 2) were observed in the nodular areas of all 21 cases. These cells had characteristically indented nuclei and cytoplasm containing distinct bundles as well as scattered 60 to 80 Å myofilaments generally aligned with the long axis of the cells. The cytoplasm also contained a well-developed system of rough endoplasmic reticulum as well as an extensive distribution of free ribosomes. Centrioles (Fig. 3) were observed in all 21 samples and in approximately 25% of the myofibroblasts seen in each of these samples. The highly cellular areas of the nodule myofibroblasts were noted to be attached to one another by junctional membrane complexes with hemidesmosomes.

On the light microscopic level, cells stained a dark brown (Fig. 4) displayed a positive reaction for ATPase at pH 7.2. When serial sections of these were examined ultrastructurally, a positive reaction precipitate for ATPase at pH 7.2 was consistently observed on the myofilaments within the myofibroblasts (Fig. 5, *A*). When ATP was omitted from the incubation medium, myofilaments did not reveal a reaction precipitate (Fig. 5, *B*).

Alcian blue staining at pH 6.5 on the light level revealed a positively stained blue matrix. On the ultrastructural level, a glycosaminoglycan matrix was also observed. This 300 to 1000 Å matrix formed connections between cells (Fig. 6) and from cells to collagen (Fig. 7). Further 100 Å attachments between the collagen fibrils were noted.

The ultrastructural characteristics of the nodular myofibroblast along with its relationships to the surrounding collagen are schematically summarized in Fig. 8.

Proximal cord of Dupuytren's disease. The cells in the cord area were elongated, resembling mature fibroblasts and aligned parallel to the long axis of collagen fibers. Their cytoplasm contained small aggregates of myofilaments; however, bundles of myofilaments were not observed. There was a paucity of cells in the cord, with most of the tissue being composed of collagen fiber bundles. These cells had a negative reaction for ATPase at all pH levels and a diminished amount of

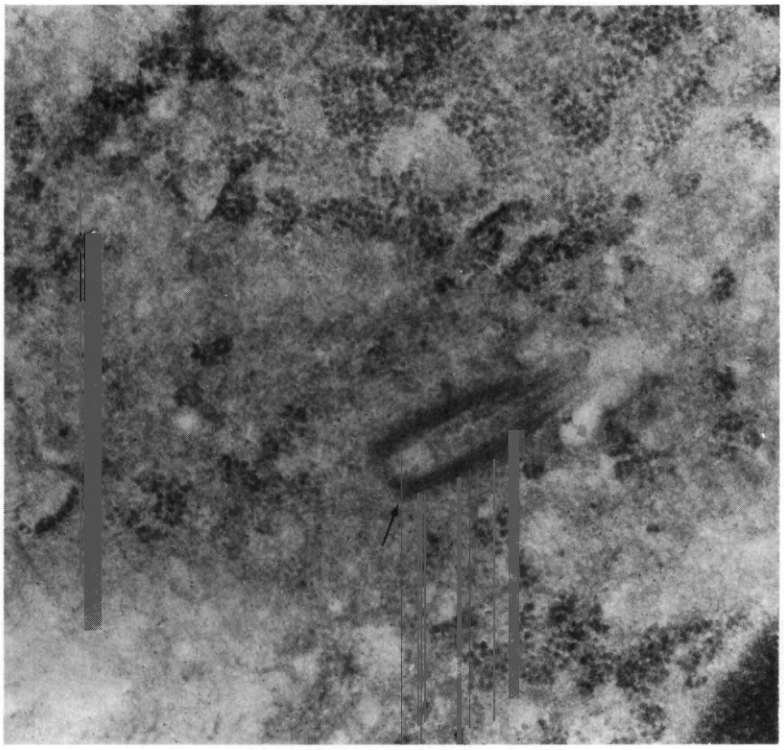

Fig. 3. Electron micrograph (×40,000) of a nodular myofibroblast. A centriole is shown within the cell cytoplasm *(arrow)*.

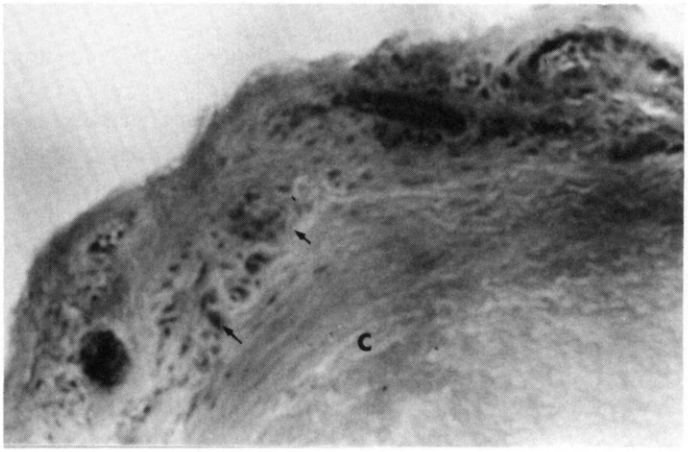

Fig. 4. Photo micrograph (×500) of a nodule. Cells *(arrows)* that are positively reactive for ATPase at pH 7.2 appear dark; collagen fibers *(C)* are light.

stained alcian blue glycosaminoglycan when compared to the nodule.

Normal palmar fascia. Fibroblasts comprised the major cell population in this tissue. In each of the six control samples small filaments were observed within the cytoplasm. Bundles of filaments were not noted within the fibroblasts. These cells displayed a negative reaction for ATPase at all pH levels and a markedly decreased alcian blue stain (Fig. 9).

Clinical-pathological correlation. Cells in the nodular areas that displayed a positive reaction precipitate for ATPase at pH 7.2 were counted in 11 patients cho-

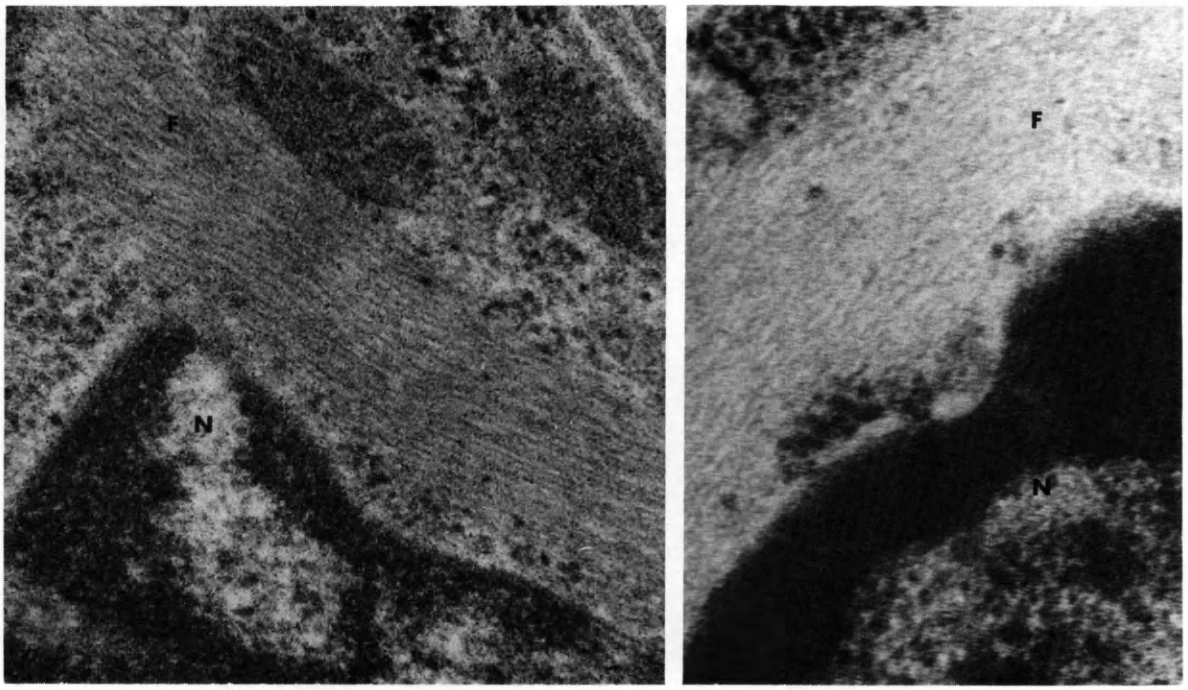

Fig. 5. Left, Electron micrograph (×100,000) of a nodular myofibroblast. A positive reaction precipitate for ATPase at pH 7.2 appears in the form of fine dots on myofilaments *(F)*. The nucleus *(N)* of the cell is evident. **Right,** Electron micrograph (×102,000) of a nodular myofibroblast. When ATP was omitted from the incubation medium to serve as a control, no reaction precipitate appears on myofilaments *(F)*. The nucleus *(N)* of this cell is evident.

sen at random. Cell counts were performed with light microscopy at a magnification of 160×. The cell counts from each of the specimens chosen were statistically correlated by the Spearman rho test to the predicted residual clinical contractures as indicated by the Legge and McFarlane Outcome Standard Formula; the Spearman rho statistical test is a nonparametric method for noninterval scales such as those seen in our data.[20, 21] The Spearman rho correlation coefficient r value ranges from −1 to +1, with +1 representing a perfect correlation. The r value correlating ATPase cells with the predicted residual contracture in our study was 0.725, which was significant at $p < .025$, indicating a statistically significant correlation between the number of ATPase active cells and the residual postsurgical clinical contractures (Fig. 10).

Discussion

These studies further substantiate the theory that myofibroblasts may play a significant role in the pathogenesis of Dupuytren's contracture. While the origin of myofibroblasts is still a matter of debate, it is noteworthy that intracellular filaments were observed in all six of our control samples. It is well established that mammalian cells such as fibroblasts contain intracellular filaments that are part of their cytoskeleton[22] and are composed of actin.[23] Therefore, it may be speculated that fibroblasts can modulate in order to form myofibroblasts. This modulation may be possible because the genome responsible for the production of contractile proteins is already present in these fibroblasts. In addition, centrioles were observed in all 21 Dupuytren's samples. The presence of these organelles suggests that these cells are capable of self-replication. It is well established that one of the functions of centrioles is that they form microtubular spindles upon which chromosomes move during the process of mitosis.[24]

The isolation of a pH-dependent ATPase further substantiates the importance of myofibroblasts to the pathogenesis of this disease. This enzyme is necessary for the process of cellular contraction and has also been observed in granulation tissue myofibroblasts by Majno et al.[3] Much attention has also been given to the collagen biochemistry of this disorder. However, the clinical characteristics of Dupuytren's disease have not been correlated to the presence of type III collagen.[10, 14] Therefore, it appears that the myofibroblasts may be significant to the development of these contractures.

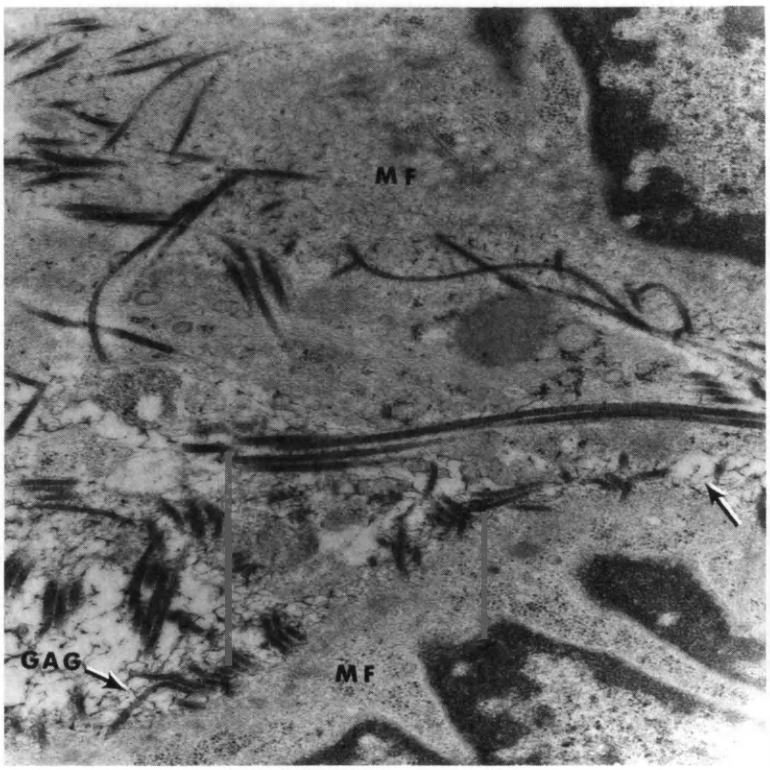

Fig. 6. Electron micrograph (×19,000) of a nodular area stained by alcian blue at pH 6.5. Myofibroblasts *(MF)* are connected to each other by a glycosaminoglycan matrix *(GAG, arrows)*, consisting of thread-like material 300 to 1000 Å thick.

The residual clinical severity, as defined by the Outcome Standard Formula,[21] was statistically correlated to the ATPase activity of the myofibroblasts. The Spearman rho correlation coefficient of 0.725 demonstrates that the ATPase active myofibroblasts can be statistically correlated to the projected residual clinical deformity. This finding is consistent with the work of Gelberman et al.,[14] which suggests a possible relationship between the magnitude of the myofibroblast population and the recurrence of the contracture.

If myofibroblasts are related to the pathogenesis of Dupuytren's contracture, then interconnections between the myofibroblasts and between the myofibroblasts and the surrounding collagen are essential. Increased proportions of glycosaminoglycans have been noted by many investigators.[5, 7, 10, 15–17] In this study the presence and ultrastructural relationships of these glycosaminoglycans to the myofibroblast and to the surrounding collagen has been clearly demonstrated by the alcian blue electron microscopic evaluation.

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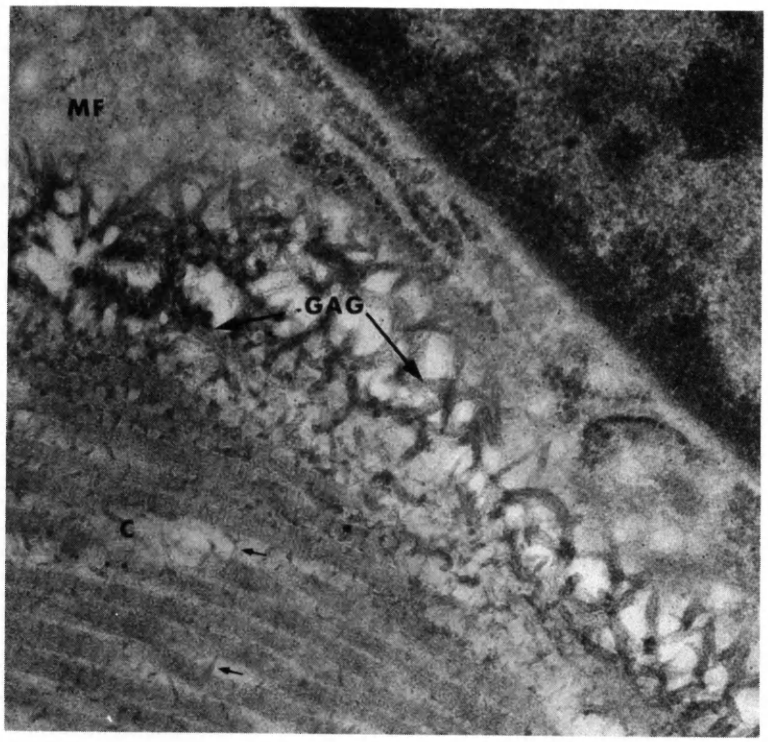

Fig. 7. Electron micrograph (×46,000) of a nodular myofibroblast *(MF)* stained by alcian blue at pH 6.5. The cell surface is attached to neighboring collagen by a glycosaminoglycan matrix *(GAG, large arrows)* 300 to 1000 Å thick. Matrix fibrils 100 Å thick connect collagen fibrils *(C)* to each other *(small arrows)*.

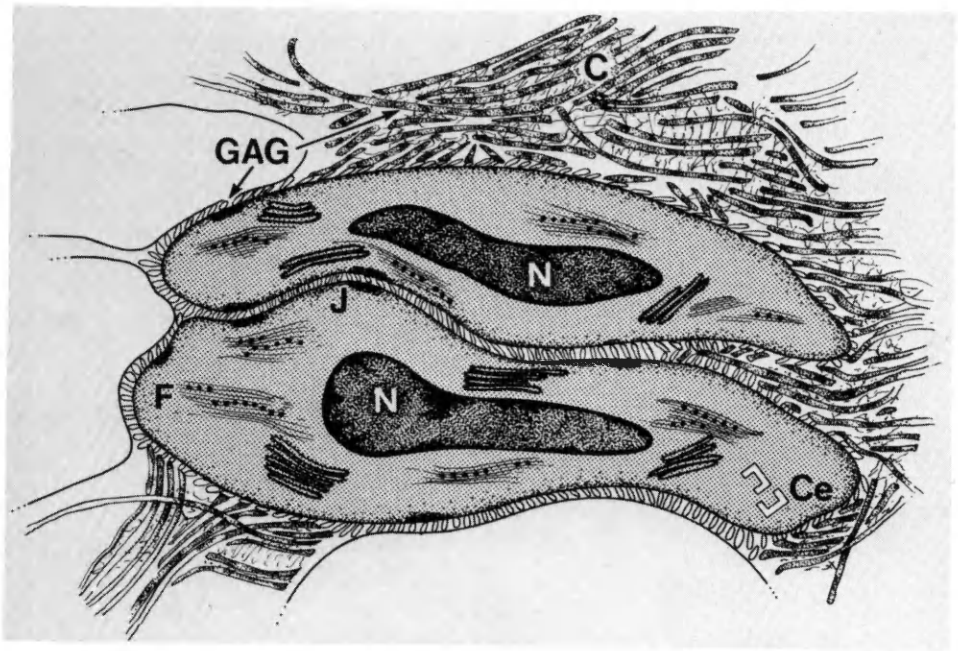

Fig. 8. Schematic drawing of nodular myofibroblasts and their ultrastructural relationships to surrounding collagen. Myofilaments with ATPase reaction precipitate *(F)*, collagen *(C)*, junction membrane complex *(J)*, glycosaminoglycans *(GAG, arrows)*, centrioles *(CE)*, and nucleus *(N)*.

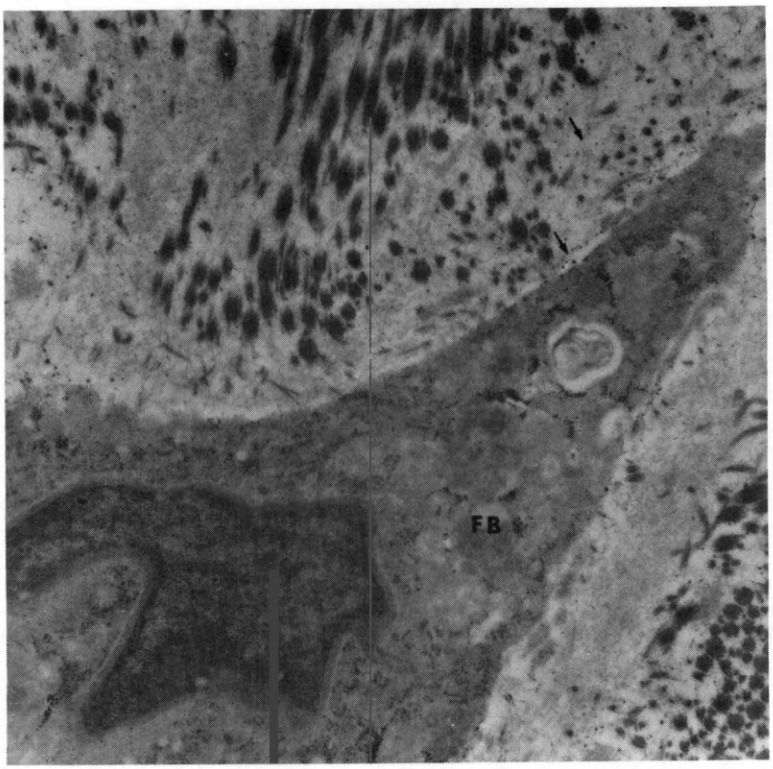

Fig. 9. Electron micrograph (×17,000) of normal palmar fascia stained by alcian blue at pH 6.5. The cell surface and surrounding matrix of this fibroblast *(FB)* show a markedly decreased staining reaction *(arrows)* for glycosaminoglycans.

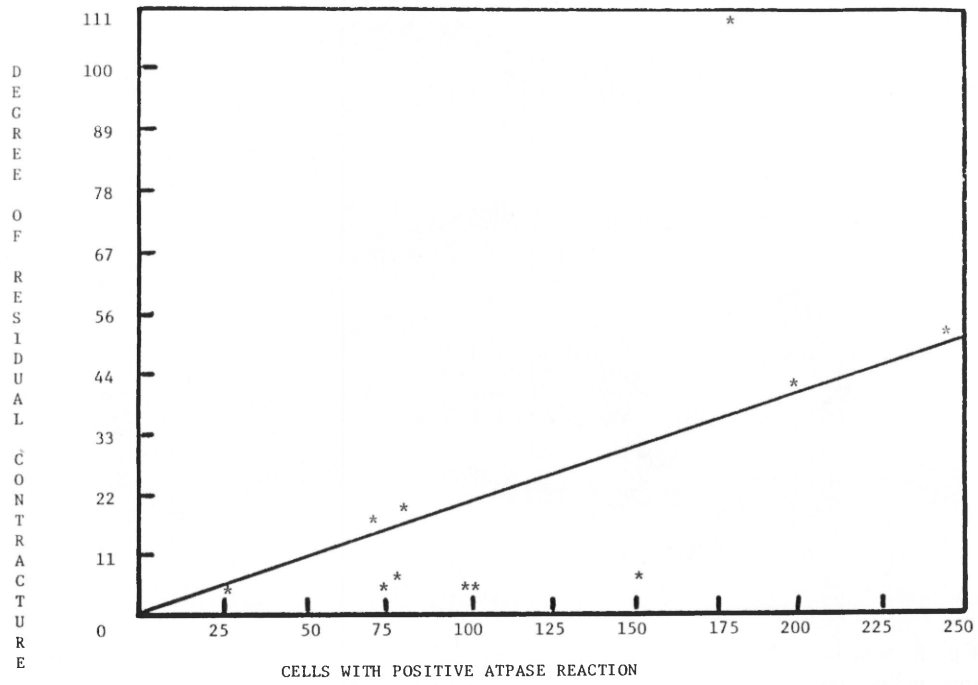

Fig. 10. Graph of ATPase activity vs degree of residual contracture as determined from the Outcome Standard Formula.

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Surgical availability of the plantaris tendon

The plantaris tendon is valuable as a donor tendon in hand surgery, but its presence is difficult to predict. Dissection of 658 cadavers showed that the tendon was present in 81.8% of limbs dissected and that there was no significant difference in the availability of plantaris between the two sexes or the sides. The incidence of bilateral absence shows that when the plantaris is absent on one side, the chance of finding it on the other is 1 in 3. There is no significant relationship between the condition of palmaris longus and the presence or usefulness of plantaris. (J HAND SURG 8:243-7, 1983.)

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The length, lateral elasticity, and dispensability of the plantaris tendon make it a suitable donor tendon for grafting. Being a rudimentary muscle like palmaris longus, it is subject to frequent minor variations and is sometimes entirely absent.[1, 2] It has sometimes been postulated that an absence of plantaris may be associated with agenesis of palmaris longus. P.W. Brand stated, "For many years, I did not even look for a plantaris in patients who had no palmaris" (personal communication, May 1979). Plantaris, unlike the palmaris longus, is usually neither visible nor palpable through the intact skin.

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