

# The pathobiology of Dupuytren's contracture: Effects of prostaglandins on myofibroblasts

The *in vitro* response of myofibroblasts to prostaglandins  $F_{2\alpha}$  (a vasoconstrictor) and  $E_2$  (a vasodilator) were evaluated in specimens obtained from the Dupuytren's nodules of 12 patients. Fibroblasts from four control samples of palmar fascia were similarly tested. This study demonstrated the ability of prostaglandin  $F_{2\alpha}$  to induce significant contraction of myofibroblasts. Prostaglandin  $E_2$  was noted to cause significant relaxation of myofibroblasts. The contractile/relaxation responses of control fibroblasts to these prostaglandins were minimal. (J HAND SURG 11A:18-22, 1986.)

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Many authors have suggested that the source of the active contractile force in Dupuytren's contracture may be the myofibroblast.<sup>1-4</sup> Gabbiani and Majno<sup>1</sup> were the first to report that the fascia of Dupuytren's disease contains nodules composed of myofibroblasts. The potential for contraction in these cells is suggested by the following ultrastructural characteristics: (1) bundles of 40 to 80 Å fibrils within the cell cytoplasm that are oriented parallel to the long axis of the cell, (2) a deeply indented nucleus, (3) cell-to-cell and cell-to-stroma membrane attachment sites known as desmosomes and hemidesmosomes. Based on these morphologic features, myofibroblasts are considered to possess properties of smooth muscle cells, as well as fibroblasts. Myofibroblasts have also been identified in other fibrotic tissues associated with the process of contraction. Examples of this are: wounds, pyogenic granuloma, ischemia contracture of the intrinsic muscles of the hand, and burn contractures.<sup>5</sup>

The major biochemical changes in Dupuytren's disease include increased amounts of type III collagen, which is usually absent from normal adult palmar fascia.<sup>6-12</sup> The hexosamine content is also increased in

affected fascia.<sup>6</sup> Increased amounts of hydroxylysine, increased numbers of reducible cross-links, and the presence of hydroxylysinohydroxynorleucine have been found in Dupuytren's fascia.<sup>6</sup> Japanese investigators have recently verified these findings. They also demonstrated a new cross-linking amino acid, pyridinoline, in Dupuytren's fascia. However, pyridinoline was found in equal amounts in normal fascia.<sup>12</sup> Recently, it has been shown that biochemical abnormalities are more prevalent in the grossly affected fascia than in the normal appearing fascia from the same hand.<sup>13</sup> However, the correlation between the severity of the clinical contractures and a quantitative estimate of these biochemical changes has not been made.

An alternative explanation may be that the contracture of the palmar fascia is not due to shortening of type III collagen fibrils, but to an active cellular process that progressively draws the distal parts of the affected tissue closer together as the original collagen is replaced by type III collagen. This hypothesis suggests that the process is quite similar to the active stages of wound repair. The factors that modulate this active cellular process are not well known.

The work of Gabbiani et al.<sup>14</sup> has addressed this question in a granulation tissue culture model. Their data show that when pharmacologically tested, myofibroblasts behave similarly to smooth muscle cells. *In vitro*, myofibroblasts contract when stimulated by various substances such as serotonin or bradykinin. We hypothesized that endogenous smooth muscle agonists might exert similar effects on myofibroblasts obtained from Dupuytren's fascia. Therefore, this study of the effects of prostaglandins (PG) $E_2$  (a smooth muscle di-

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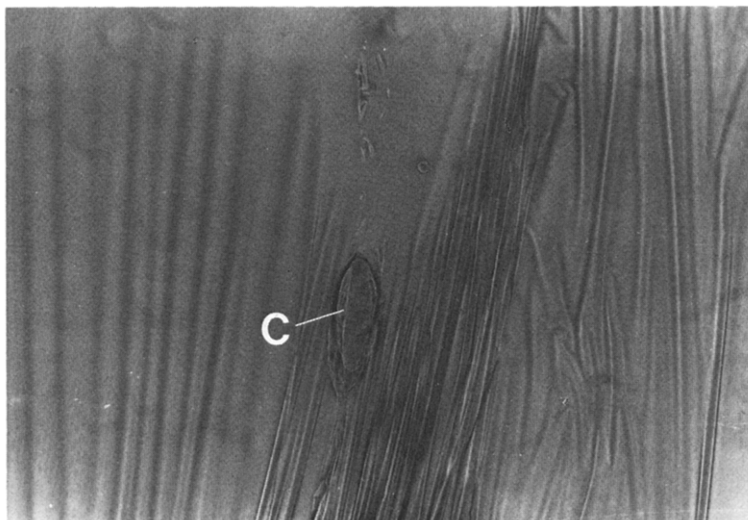
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**Fig. 1.** Photomicrograph of cell aggregates in culture resting on the silicone substrate. Cells (*c*) are shown to be attached to the substrate that has wrinkled (*w*) in response to the baseline forces on it. (Original magnification  $\times 200$ .)



**Fig. 2.** Photomicrograph of untreated Dupuytren's cell aggregate (*c*) attached to silicone substrate. (Original magnification  $\times 200$ .)

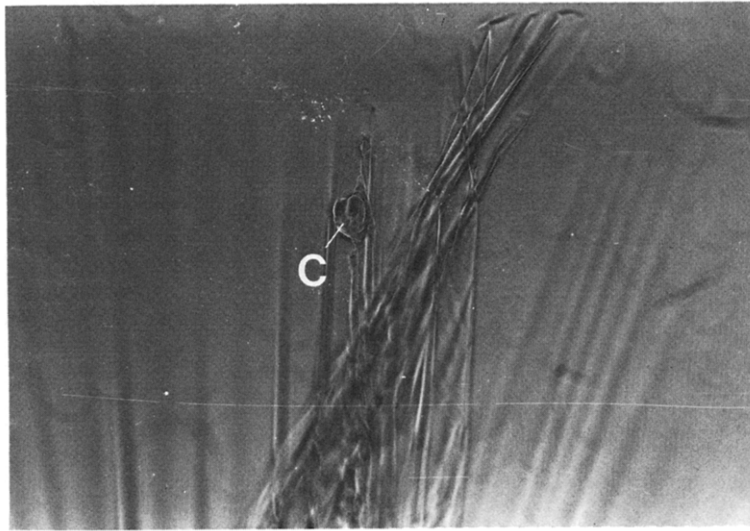
alator) and  $(PG)F_{2\alpha}$  (a smooth muscle constrictor) on cultured myofibroblasts was undertaken.

### Methods

Nodules were obtained from 12 patients with Dupuytren's contracture of the hand who were treated by regional fasciectomy. Control samples of palmar fascia were obtained from four patients having hand surgery for other reasons.

Surgical samples were established in culture with Eagle's minimal essential medium containing 10% fetal calf serum and 0.1% penicillin streptomycin at 37° C. Before addition of the medium, the bottoms of the culture dishes were lined with sterile cover slips on which a transparent 1  $\mu$ m silicone substrate had been placed. This substrate has the ability to wrinkle and relax in response to forces on it.<sup>15</sup>

After a 7- to 10-day growth interval, cultured cells,



**Fig. 3.** Photomicrograph of Dupuytren's cell aggregate (*c*) from Fig. 2. One mg/ml  $\text{PGF}_{2\alpha}$  was added to the culture medium. Cells are significantly contracted from the untreated condition. (Original magnification  $\times 200$ .)

which were attached to the substrate, were photographed at constant magnification by phase contrast microscopy. Thereafter,  $\text{PGF}_{2\alpha}$  was added to the medium followed by  $\text{PGE}_2$  while under direct phase contrast observation. The concentration of prostaglandins used included: 1.0  $\mu\text{g}/\text{ml}$ , 0.25 mg/ml, 0.50 mg/ml, or 1.0 mg/ml. Photographs of cell aggregations (Fig. 1), which were attached to the substrate, were quantitated at time zero and after the addition of prostaglandins, approximately 60 to 90 seconds later, by serial measurements of their diameter with a computer-assisted image analyzer.

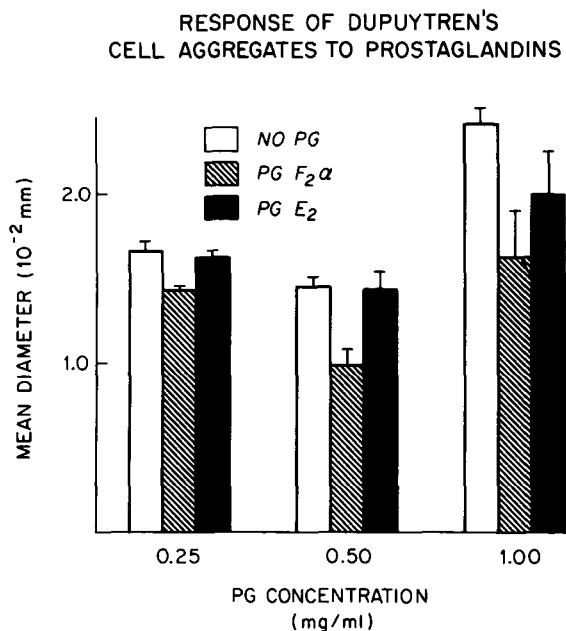
Cells from culture were subsequently prepared for transmission electron microscopy to confirm that they were either myofibroblasts or fibroblasts. The cover slips with attached cells and substrate were treated as distinct specimen samples. These were fixed in 3.0% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, for 1 hour at 4° C. After a cold buffer rinse, samples were postfixated with 1.0% osmium tetroxide for 1 hour at 4° C. Dehydration was accomplished in an ascending ethanol series followed by clearing in propylene oxide, with infiltration in 3:1 propylene oxide to Polybed 812 resin (Polysciences, Warrington, Pa.). Samples were embedded at 60° C for 24 hours in specialized flat embedding molds that securely fit the length, width, and depth of the cover slip. Embedded samples were transferred by gluing to blank specimen blocks, sectioned on a Sorvall MT2B-ultramicrotome with a diamond knife at 400 Å, stained in uranyl acetate and lead

citrate, and photographed in a Hitachi HU-12 electron microscope.

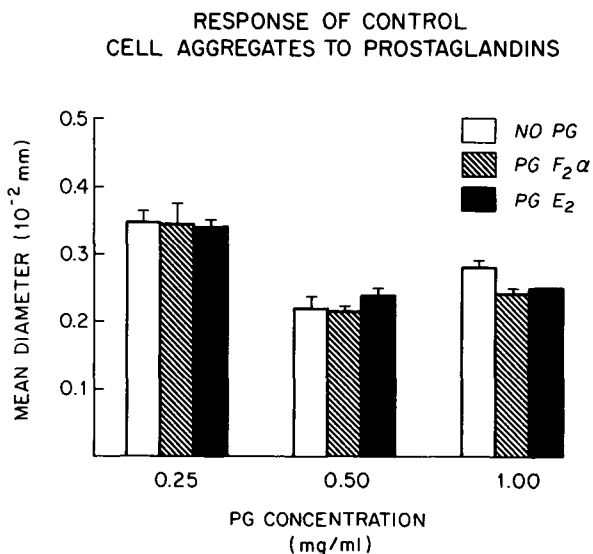
### Results

$\text{PGF}_{2\alpha}$  was noted to induce contraction of cell aggregates, while  $\text{PGE}_2$  caused relaxation of aggregates on the silicone substrate at all prostaglandin concentrations used except 1.0  $\mu\text{g}/\text{ml}$ . The relative degree of contraction or relaxation was calculated by comparing the percentage of change in the diameters of *untreated* cell aggregates (Fig. 2) in culture to those treated (Fig. 3) with prostaglandins. As an internal control, before the addition of prostaglandins to the culture medium, cover slips supporting cell growth were bathed in pure medium under direct microscopic observation to ensure that cells were firmly attached to the substrate and that they did not respond in any way to the addition of medium alone.

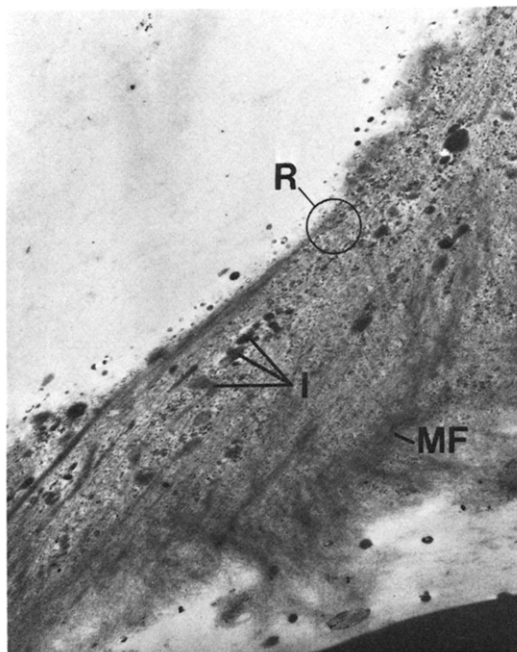
Cells cultured from Dupuytren's nodules exhibited a 26% relative contraction response to  $\text{PGF}_{2\alpha}$ . That is, the average serial diameters of the cell aggregates were reduced by 26% by  $\text{PGF}_{2\alpha}$ . Thereafter, when  $\text{PGE}_2$  was applied to these contracted aggregates, the diameters increased to a value that was approximately 7% less than the original untreated cell aggregates (Fig. 4). Therefore, the 26% relative contraction response, caused by  $\text{PGF}_{2\alpha}$ , was reduced by 19% when the  $\text{PGE}_2$  was added. Cells from control cultures exhibited less than a 1% relative contraction/relaxation response when subjected to  $\text{PGF}_{2\alpha}$  and then  $\text{PGE}_2$  (Fig. 5).



**Fig. 4.** Contraction/relaxation responses of Dupuytren's cell aggregates (myofibroblasts) to prostaglandins in vitro. Mean diameters decreased from untreated values in response to PGF<sub>2α</sub>. Mean diameters increased when PGE<sub>2</sub> was added to contracted aggregates. Standard deviations appear above each bar. Two-tailed *t* test for level of significance  $p < 0.001$  for PGF<sub>2α</sub> response and  $p > 0.025$  for PGE<sub>2</sub> response.



**Fig. 5.** Contraction/relaxation responses of control cell aggregates (fibroblasts) to prostaglandins in vitro. Minimal responses were noted by the addition of prostaglandins. Standard deviations appear above each bar. Two-tailed *t* test for level of significance  $p < 0.001$  for PGF<sub>2α</sub> response and  $p < 0.001$  for PGE<sub>2</sub> response.



**Fig. 6.** Electron micrograph of a cultured myofibroblast from a Dupuytren's nodule. Bundles of intracellular filaments (MF) are shown. Ribosomes (R) and amorphous inclusions (I) are seen within the cell. (Original magnification  $\times 50,000$ .)

Transmission electron microscopy confirmed the presence of myofibroblasts in the cultures from Dupuytren's nodules. Fig. 6 denotes the parallel arrays of filaments within the cytoplasm of these cultured cells. Cultured fibroblasts from control samples did not contain any bundles of intracytoplasmic filaments.

### Discussion

This data suggest that prostaglandins F<sub>2α</sub> and E<sub>2</sub> can induce contraction and relaxation, respectively, of myofibroblasts from Dupuytren's palmar fascia in vitro. In vivo, prostaglandins may be regionally produced and exert their physiologic effects at nanogram and picogram concentrations. Because of the characteristics of this model, which necessitated the diffusion of the prostaglandins through culture media, in which silicone-coated cover slips were suspended, larger doses of prostaglandins were necessary to produce optically demonstrable changes in cell aggregate diameters. In their work on the response of granulation tissue myofibroblasts to serotonin, Gabbiani et al.<sup>14</sup> also used doses at higher physiologic levels.

The literature has documented decreased PGE levels in both animals and humans in response to chronic ethanol exposure and chronic intake of antiseizure

medications such as diphenylhydantoin.<sup>16-18</sup> Chronic alcoholism<sup>19-21</sup> and epilepsy under treatment with antiseizure medications<sup>20, 22</sup> are conditions that are known to be clinically associated with Dupuytren's contracture. Direct quantitation of PGE levels in patients with Dupuytren's contracture is necessary to substantiate the hypothesis; however, relaxation of myofibroblasts in Dupuytren's disease may be inhibited by decreased PGE<sub>2</sub> levels.

A possible mechanism implicating PGE<sub>2</sub> is the documented inhibition of adenosine triphosphatase (ATPase) by this prostaglandin.<sup>23</sup> In muscle, ATPase is associated with the two globular thick filament myosin heads. During contraction, ATPase splits high-energy ATP bonds. Subsequently, each myosin globular head is attracted by a succession of calcium-active actin sites resulting in a sliding of the action filaments into the spaces between the myosin filaments.<sup>24</sup> Thus, if ATPase is inhibited, contraction will not occur. Our previous study has confirmed that ATPase is present in the intracellular filaments of the myofibroblasts.<sup>3</sup> Thus, PGE<sub>2</sub>'s ability to relax the myofibroblasts in vitro in the present study may occur through the mechanism of ATPase inhibition within the myofibroblasts; that is, prostaglandin E<sub>2</sub> is relaxing the myofibroblast by inhibiting ATPase.

This in vitro study has shown that cultured myofibroblasts contract when exposed to PGF<sub>2α</sub>. This is consistent with the known vasoconstrictive action of PGF<sub>2α</sub>. Additionally, PGF<sub>2α</sub> is known to be associated with increased synthesis of hexosamine glycosaminoglycans.<sup>25</sup> It is well established that there are increased amounts of hexosamine glycosaminoglycans present in the fascia of Dupuytren's disease.<sup>3, 6-11</sup> Prostaglandin E also inhibits ATPase, which may be an important consideration in myofibroblast contraction. Furthermore, prostaglandin E is decreased in alcoholism and patient's receiving anti-seizure medication. The association of Dupuytren's disease with alcoholism and epilepsy may be related to prostaglandin metabolism.

## REFERENCES

- Gabbiani H, Majno G: Dupuytren's contracture: fibroblast contraction. An ultrastructural study. *Am J Pathol* 66:131-8, 1972
- McFarlane RM: *In* Green DP, editor: Operative hand surgery. New York, 1982, Churchill Livingstone, p 463
- Badalamente MA, Stern L, Hurst L: The pathogenesis of Dupuytren's contracture: contractile mechanisms of the myofibroblasts. *J HAND SURG* 8A:235-42, 1983
- VandeBerg JS, Gelberman RH, Rudolph R, Johnson D, Sicirello P: Dupuytren's disease: comparative growth dynamics and morphology between cultured myofibroblasts (nodule) and fibroblasts (cord). *J Orthop Res* 2:247-56, 1984
- Seemayer TA, Lagace R, Schurch W, Thelmo WL: The myofibroblast: biologic, pathologic and theoretical considerations. *Pathol Ann* 1:443-70, 1980
- Brickley-Parsons D, Glimcher M, Smith R, Albin R, Adams J: Biochemical changes in the collagen of the palmar fascia in patients with Dupuytren's disease. *J Bone Joint Surg [AM]* 63:787-97, 1981
- Gelberman R, Amiel D, Rudolph R, Vance R: Dupuytren's contracture. Electron microscopic, biochemical and clinical correlative study. *J Bone Joint Surg [AM]* 62:425-32, 1980
- Bazin S, LeLous M, Duance V, Sims T, Bailey A, Gabbiani G, D'Andiran G, Pizzolato G, Browski A, Nicoletis C, Delaunay A: Biochemistry and histology of the connective tissue of Dupuytren's lesions. *Eur J Clin Invest* 10:9-16, 1980
- Baily A, Sims T, Gabbiani G, Bazin S, LeLous M: Collagen of Dupuytren's disease. *Clin Sci* 53:499-502, 1977
- Menzel E, Piza H, Zielinski C, Endler A, Steffen C, Millesi H: Collagen types and anti-collagen antibodies in Dupuytren's disease. *Hand* 11:243-8, 1979
- Hunter J, Ogdon C, Norris M: Dupuytren's contracture. I. Chemical pathology. *Br J Plast Surg* 28:10-18, 1975
- Hanya T, Tajima T, Takagi T, Sasaki S, Fujimoto D, Isemura M, Yosizawa Z: Biochemical studies on the collagen of palmar aponeurosis affected with Dupuytren's disease. *Tohoku J Exp Med* 142:437-43, 1984
- Parsons D, Adams S, Smith R, Glimcher MJ: Collagen polymorphism in Dupuytren's disease. *Trans Orthop Res Soc* 10:116, 1985
- Gabbiani G, Hirschel BJ, Ryan GB, Statkov PR, Majno G: Granulation tissue as a contractile organ. A study of structure and function. *J Exp Med* 135:719-34, 1972
- Harris AK, Wild P, Stopak D: Silicone rubber substrata: a new wrinkle in the study of cell locomotion. *Science* 208:177-9, 1972
- Horrobin DF: A biochemical basis for alcoholism and alcohol-induced damage including the fetal alcohol syndrome and cirrhosis: interference with essential fatty acid and prostaglandin metabolism. *Med Hypotheses* 6:929-42, 1980
- Pennington SN, Smith CP, Strider JB: Alterations in prostaglandin catabolism in rats chronically dosed with ethanol. *Biochem Med* 21:246-52, 1979
- Tobinson DR: Prostaglandins and the mechanism of action of anti-inflammatory drugs. *Am J Med* 75(suppl): 26-31, 1983
- Hueston JT: *In* Flynn JE, editor: Hand surgery, ed 3. Baltimore, 1982, Williams & Wilkins Co, pp 797-9
- Pojer J, Radivojevic M, Williams F: Dupuytren's contracture. Its association with abnormal liver function in alcoholism and epilepsy. *Arch Intern Med* 129:561-6, 1972

21. Nazari B: Dupuytren's contracture associated with liver disease. *J Mt Sinai Hosp NY* 33:69-72, 1966
22. Critchley EMR, Vakil SD, Hayward HW, Owen VMH: Dupuytren's disease in epilepsy: result of prolonged administration of anticonvulsants. *J Neurol Neurosurg Psychiatry* 39:498-503, 1976
23. Hall AK, Behrman HR: *In Lee JB, editor: Prostaglandins*. New York, 1982, Elsevier Science Publishing Co, Inc, pp 1-38
24. Ebashi S, Endo M, Ohtsuki I: Control of muscle contraction. *Rev Biophys* 2:351-84, 1969
25. Hall AK, Behrman HR: *In Lee JB, editor: Prostaglandins*. New York, 1982, Elsevier Science Publishing Co, Inc, pp 91-112

## Surgery of Dupuytren's disease: A review of the open palm method

**Experience with 49 cases of Dupuytren's disease treated by the open palm method and followed for an average of 5 years is reported. This method permits excision of the involved fascia while minimizing early postoperative morbidity associated with the surgical management of this condition. There were no cases of wound necrosis, hematoma, or infection with this technique, and all wounds closed spontaneously within 3 to 5 weeks. Long-term follow-up, however, revealed a significant rate of recurrence (32%) and extension (48%), as well as flexion loss at one or more joints in 41% of involved hands. While helpful in the early care of these patients, the open palm method does not modify the long-term results in Dupuytren's disease, which is a pervasive condition without simple surgical solution. (J HAND SURG 11A:23-27, 1986.)**

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Dupuytren's disease continues to pose treatment problems for both patient and surgeon. Many approaches and techniques are available when the deformity is significant enough to warrant surgery. The goals of any treatment program should include correction of the deformity and restoration of hand function. Early complications of surgical treatment have included hematoma, wound necrosis, infection, and reflex sympathetic dystrophy. Late problems include joint stiffness, scar contractures, recurrence of the disease in the operated area, and extension of the disease into previously uninvolved areas. To minimize the early prob-

lems associated with surgery in these patients, the open palm technique modified from McCash has been used on our service in selected patients for the last 14 years.

McCash<sup>1</sup> presented his technique of management in Dupuytren's disease in 1964. In this technique, he used a series of incisions in the transverse creases of the palm and fingers, removed the diseased fascia, and then closed all the incisions except the one at the distal palmar crease. Early motion was started at the first dressing change, which was done at 1 week. In his article, McCash credits the concept of open treatment to Dupuytren<sup>2</sup> himself. Others have written about their experiences with the open method,<sup>3-14</sup> and all concur regarding the advantages of this technique in the early management of these patients. However, none have presented a long-term review of their cases. Two series of cases have been published as comparison studies in which closed treatment was evaluated against the open method in an effort to weigh the advantages of each. Lubahn et al.<sup>15</sup> stated that patients in the closed group had more residual contracture than those in the open

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