Characterization of Implants from Dupuytren's Contracture Tissue into the Nude (Athymic) Mouse (42859)

C. W. KISCHER, J. PINDUR, J. MADDEN, M. R. SHETLAR, AND C. L. SHETLAR

Department of Anatomy, University of Arizona, College of Medicine, Tucson, Arizona 85724; Academy Medical Center, Tucson, Arizona 85711; and Department of Food and Nutrition and the Departments of Biochemistry and Dermatology, Texas Tech University, Health Science Center, Lubbock, Texas 79430

Abstract. **Dupuytren's contracture tissues were obtained from six patients as excess surgical material. Pieces of these tissues (a total of 38 implants) were placed into subcutaneous pockets in the suprascapular area of nude (athymic) mice. The objective was to determine whether the implant tissues would be maintained in the mouse with the characteristics of Dupuytren's tissue. The implants were removed for study at 14–179 days after implantation. Microvascular anastomosis between implant and host skin was established within the first 14 days. Histologic character and electron microscopic structure of the implants did not change during the course of the study. The implants became reduced in size with time. However, neither the spatial pattern of collagen nor the appearance of fibroblast cells changed. The original high levels of chondroitin-4-sulfate were significantly decreased in the 66- to 179-day postimplantation group, but were not significantly different from the values for normal fascial bands. The hyaluronic acid of the implants increased significantly with time of implantation, but never reached the level found in the normal fascial bands. The use of implants into nude mice may be useful for further experimental studies of Dupuytren's contracture.**

[P.S.E.B.M. 1989, Vol 190]

Dupuytren's contracture (DC) is an idiopathic disease of the palmar aponeurosis involving the fascial bands. Usually the longitudinal bands are predominantly involved, but there can be involvement with the transverse and vertical fibers (1). VandeBerg *et al.* (2) have shown that the overlying palmar skin is not involved. The origin of DC is unknown but has been variously reported to be associated with trauma, alcoholism, diabetes (arthritis), or racial-genetic factors (3–5). The morphologic character of the lesion includes nodular collagen which contains myofibroblasts (6) apparently accounting for the contracture phenomenon of the affected digits. The bands are essentially avascular, but the majority of the peripheral microvessels are occluded by an increase in their own endothelial cells which bulge into the lumen. They also have an unusual heavy layering of basal lamina about the endothelial cells (7). Biochemically, Dupuytren's tissue resembles other proliferating connective tissue, for which increased amounts of chondroitin 4-sulfate, soluble collagen, and type III collage have been reported (8, 9).

This peculiar condition apparently can be experimentally produced in monkeys (10), although it has not been reported as occurring naturally in any animal. We have implanted pieces of hypertrophic scars and keloids into athymic nude mice and carried them successfully up to 60 days (11) with little or no change in their histiotypic character or glycosaminoglycan distribution. However, additional studies have shown that originally high levels of chondroitin 4-sulfate are reduced in implants longer than 60 days (12). The hypertrophic scar and keloid are apparently also unique to man. It occurred to us, therefore, to implant pieces of Dupuytren's contractures into the nude mouse and characterize them by procedures used in the study of hypertrophic scars. Thus, the objective of this study was to determine whether the implants of DC retain their character over time. If so, they may constitute a suitable model for the experimental study of this lesion in terms of its growth, maintenance, and possible nonsurgical resolution.

Received September 1, 1988. [P.S.E.B.M. 1989, Vol 190]
Accepted November 23, 1988.

0037-9727/89/1903-0268$02.00/0

Materials and Methods

Male athymic nude mice (*nu/nu*) were obtained from the Harlan Sprague-Dawley Co. and housed in a special room with limited access. The mice were kept one to a unit in which the bedding was sterilized and over which was a filtered bonnet. The units were placed in front of laminar positive air flow shelves. All procedures were done under a protective (or laminar flow) hood under aseptic conditions.

Tissue specimens from six different patients with Dupuytren's contracture were used from which 38 implants were made into 17 mice. The implants were removed at various times ranging from 14 to 179 days: 2 at 14 days, 2 at 16 days, 4 at 27 days, 2 at 36 days, 5 at 44 days, 3 at 66 days, 4 at 69 days, 2 at 104 days, 3 at 106 days, 3 at 107 days, 2 at 109 days, 2 at 127 days, 2 at 136 days, and 2 at 179 days.

The donor tissues were obtained as excess material and consisted of contracture bands with peripheral fatty tissue. Sections were made by hand using a sterile stainless steel blade through the middle or largest part of the band. A midsection was then cut to a standardized size of approximately 5 × 8 × 5 mm. In all cases some peripheral fatty tissue was left attached to the piece for implantation. For the implantation procedure, each mouse was anesthetized with sodium pentobarbital (40 mg/kg). A full-thickness incision was made over the scapular area and a subcutaneous pocket formed with the tips of surgical scissors. The donor tissue was placed in the pocket and the skin closed with 12-mm wound clips, which were removed after 10 days (Fig. 1).

Pieces were also taken from donor tissues for the control studies. Two specimens were fixed by immersion in Karnovsky's fixative; one was used for light microscopy using hematoxylin and eosin (H & E) and Masson's trichrome staining. The other piece was processed for study by transmission electron microscopy. A third specimen was frozen for glycosaminoglycans (GAG) assays. A few normal transverse fascial bands were obtained, frozen for GAG assays, and small fresh pieces were also fixed and processed for light and transmission electron microscopy as control tissues. When the implants were removed the mouse was anesthetized as before, the skin incised, and the implants removed together with the overlying skin. Usually the implants were well fixed to the surrounding subcutaneous mouse tissue.

Each implant was measured for length, width, and thickness upon harvesting. The measurements of the pieces prior to and at the time of implantation allowed for detection of change in volume. One implant was immediately frozen for future biochemical assays and the other was placed in Karnovsky's fixative for morphologic studies.

All implants were examined grossly for a vascular supply from the overlying skin. Two of the mice were

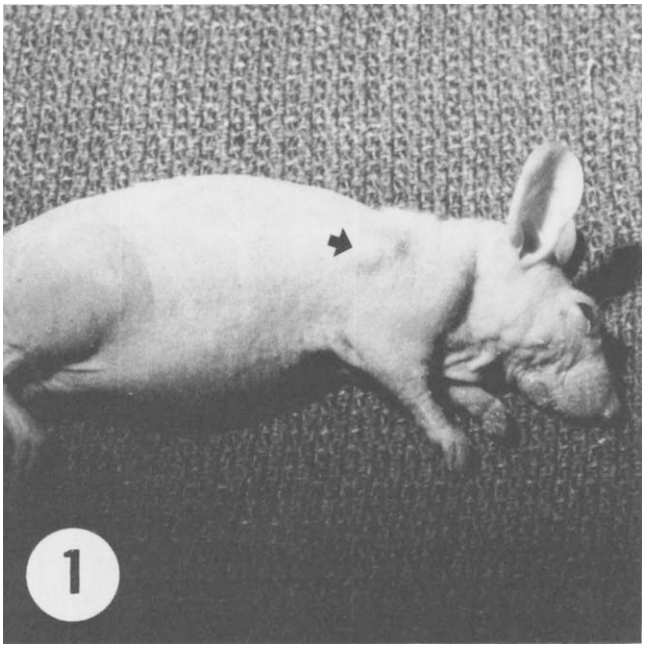

Figure 1. Mouse with implant (◗) over scapular area, 12 days after implantation.

injected with barium sulfate suspension at 69 and 106 days. For this procedure the mice were anesthetized with a intraperitoneal injection of sodium pentobarbital and tied to a cork board. The ascending aorta was cannulated via the left ventricle with a polyethylene cannula. A heparinized saline solution was first injected to reduce the possibility of perfusion obstruction. The left jugular vein was nicked to observe barium escape. The barium suspension (33 g of barium sulfate, 4 g of gelatin, 100 ml of water) was then injected with steady pressure until the mouse's systemic circulation was filled with barium and leaked from the nicked jugular vein. Upon completion of the barium procedure, the back skin with the implants were dissected away from the mouse, pinned out in a petri dish filled with dental wax, and immersed in cold Karnovsky's fixative. Serial macrosections of uniform thickness were made through the implant and overlying skin. The sections were placed on glass; microangiograms were prepared with high resolution x-rays taken of these sections for evidence of microvascular anastomosis. The other implant (on the other side) was examined by light microscopy using H & E staining. Additionally, the histologic sections of the other implants recovered were also examined (some by serial sectioning) for evidence of contiguous microvascular anastomosis.

Tissues to be examined by transmission electron microscopy were diced to appropriate size and immersed in Karnovsky's fixative for several hours. After postfixation in osmium tetroxide for 1 hr, the tissues were dehydrated in graded alcohol and embedded in Epox 812 (Fullam). Thin sections were cut with a diamond knife, stained with uranyl acetate, and ex-

amined in a Philips 300 transmission electron microscope. The transmission electron microscopic sections were assessed for confirmation of microvascular occlusion and fine structural characteristics of fibroblast-type cells.

Tissues and implants reserved for GAGS assays were weighed, thawed, and placed in an acetate buffer (0.2 *M*, pH 7) and digested with papain, followed by separation of the GAG by cellulose acetate electrophoresis as previously described (13). The amount of each GAG fraction was estimated by means of a Beckman Microzonal Densitometer model R112 and expressed as a percentage of the total GAG levels.

Results

All implants remained viable and healthy. There was no evidence of rejection, inflammation, infection, or round cell infiltration. Although the tissues cut for implants using a razor blade had sharp corners, all implants when recovered displayed rounded edges and were remodeled into elliptical shapes. Implants were covered by a thin layer of loose areolar tissue from the mouse subcutaneous layer.

The younger implants usually displayed one major vessel appearing as a branch from a subcutaneous vessel, presumably from an artery which arborized directly onto the surface of the implant (Fig. 2). In older implants more than one vessel would sometimes be seen supplying the implant. Examination of H & E sections demonstrated microvascular anastomosis in all implants, the youngest being at 14 days after implantation. Microangiograms confirmed the anastomosis (Fig. 3); these were confirmed by H & E section (Fig. 4). The periphery of the implant showed a plethora of microvessels. No increase in microvessels over zero-time tissues was noted in the rest of the implant.

All zero-time Dupuytren's tissues had active-appearing fibroblasts and all samples contained occluded

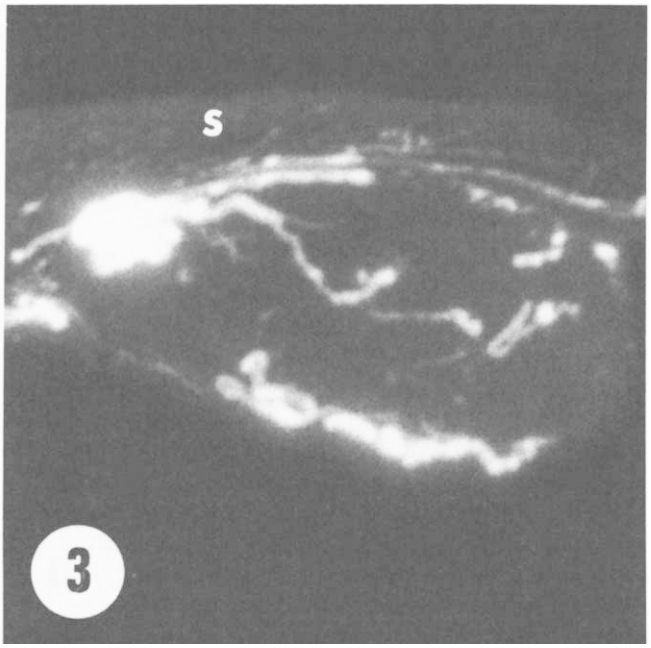

Figure 3. Microangiogram after barium sulfate injection of middle section though an implant after 106 days. Mouse skin (S) above implant. Bright areas to left and bottom represent plexi of vessels filled with barium (original magnification ×40).

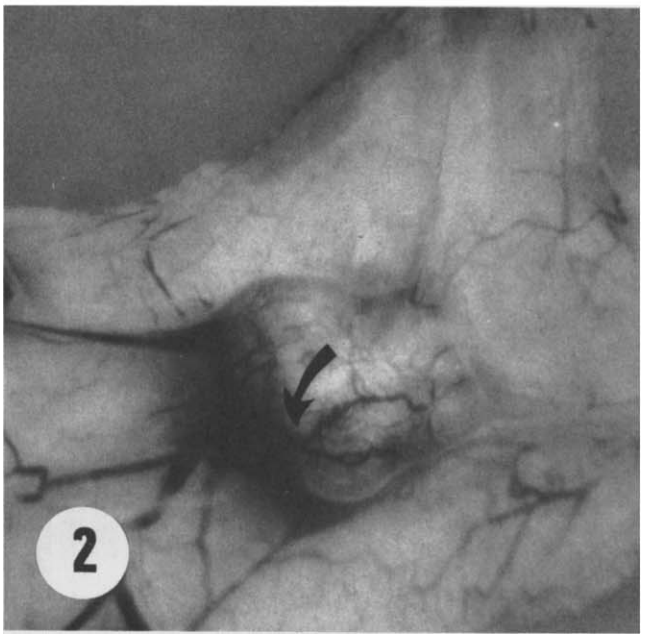

Figure 2. Implant of 11 days demonstrating arborized vessel over surface of implant (◗). Mouse skin with stem vessel to the left (original magnification ×10).

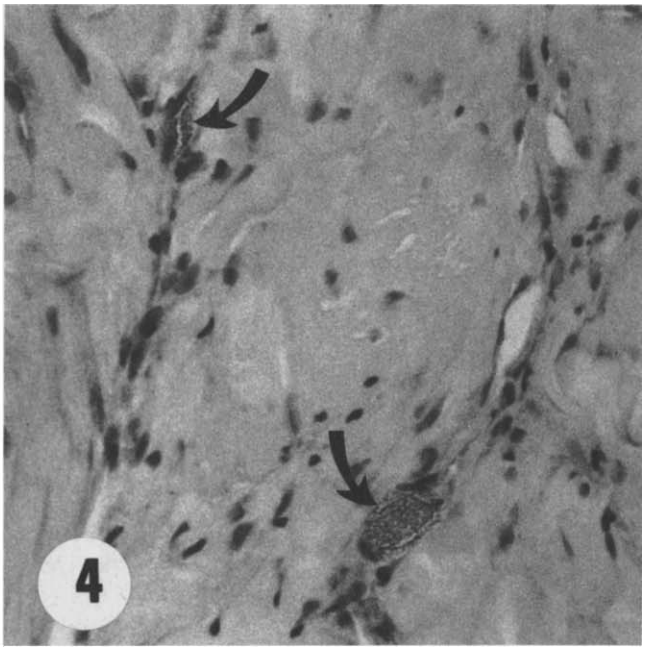

Figure 4. H & E section through implant of 69 days injected with barium. Note vessels of implant containing barium (↓) (original magnification ×325).

microvessels (Fig. 5), especially in the peripheral fatty tissue. On the other hand, transverse fascial bands used as control tissues had no occluded microvessels and no active fibroblast-type cells (Fig. 6). The collagen fasicles were relatively small and considerable interstitial space was noted.

Implants were evaluated histologically and compared with their zero-time tissues and the following

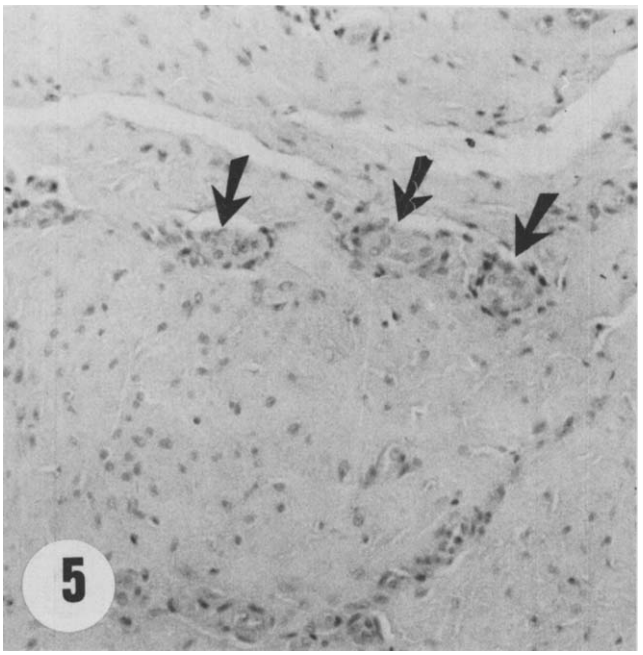

Figure 5. DC tissue from band, fresh nonimplanted. Active fibroblasts and occluded microvessels (↓) (H & E; original magnification ×200).

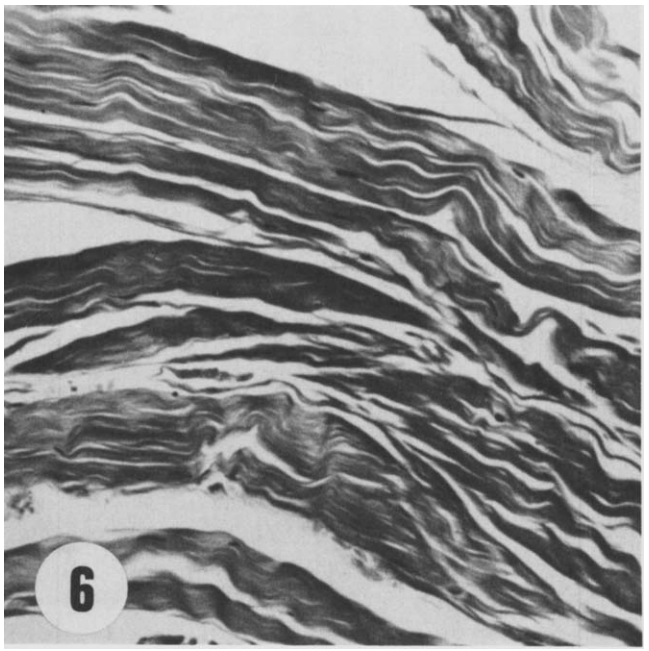

Figure 6. Normal fascial band from hand. Collagen fascicles are small with interstitial space (H & E; original magnification ×200).

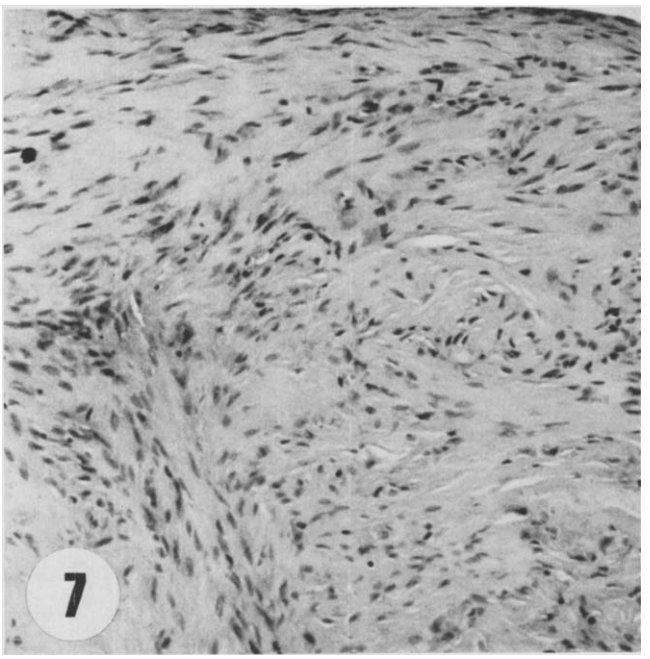

Figure 7. DC implant of 127 days with many active appearing fibroblasts (H & E; original magnification ×200).

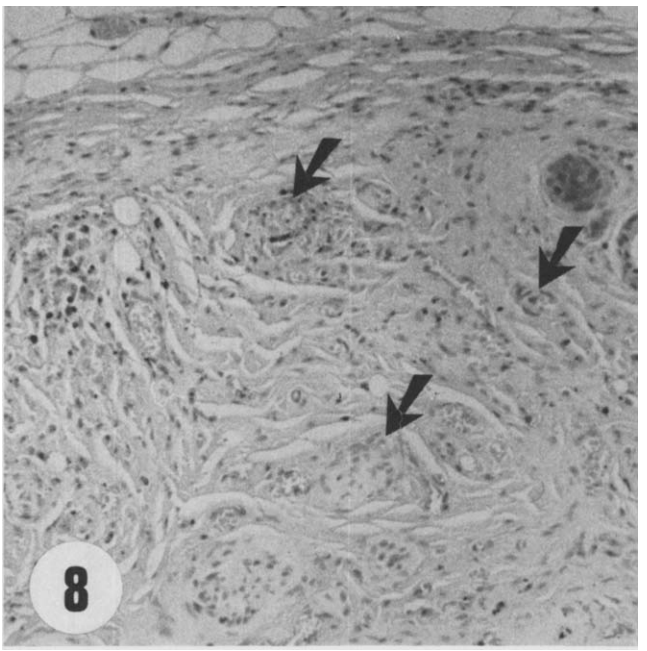

Figure 8. DC implant of 44 days with occluded microvessels (↓) (H & E; original magnification ×200).

results were obtained:

1. No change in apparent density or character of the collagen was observed for any length of implantation.

2. Only two implants, at 44 and 127 days, showed slight increases in numbers of active fibroblasts in the bands proper (or nodules) (Fig. 7), and two others, at 104 and 179 days, showed slight decreases.

3. The number of occluded microvessels remained

essentially unchanged from the zero-time assessments (Fig. 8).

The studies of the Dupuytren's tissues by transmission electron microscopy confirm that the fibroblasts identified as active by H & E sections appear active by transmission electron microscopic profile (Fig. 9). There are no essential differences among the cells in the implants compared with those in the zero-time tissues. The observation that microvessels identified as occluded by light microscopic section were confirmed by transmission electron microscopy (Fig. 10).

Twenty-seven of the implants were measured at the time of harvesting and compared with their zero-time measurements for change in volume (Table I). When these measurements were subjected to a regression analysis, a slope value was derived at −0.70152. This means that viewing the change in volume in an implant as a linear function, each implant was reduced in volume an average of about 0.70%/day; in 100 days the volume would be 30% the original volume. Actually, the average volume of the 10 implants removed at 104 to 109 days was 43 mm^3 which is 34% of the original. None of the implants demonstrated a change in their histologic character. The distribution and architectural pattern of the collagen which characterizes the contracture band is sustained, even when the time

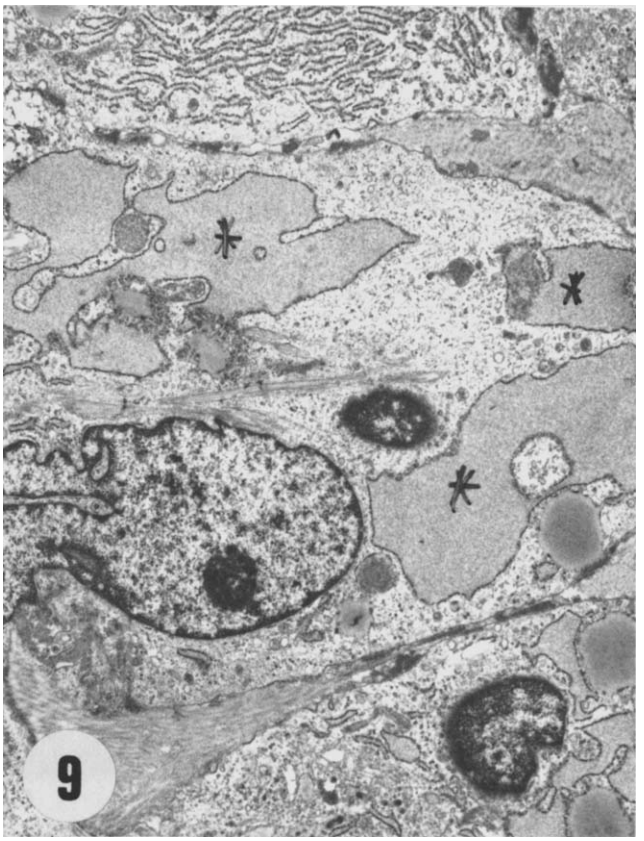

Figure 9. Electron micrograph of active fibroblasts as evidenced by dilated rough endoplasmic reticulum (*) in band from implant of 44 days (original magnification ×6700).

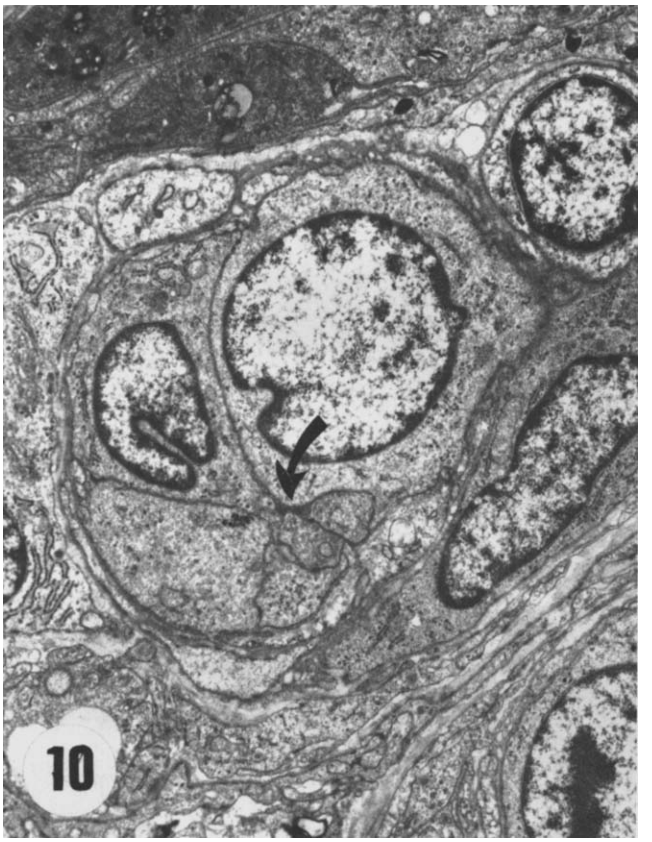

Figure 10. Electron micrograph of occluded microvessel in band from implant of 107 days. Occluded lumen (↓) surrounded by profiles from at least six endothelial cells (original magnification ×6700).

of implantation was 179 days. The reduction in size, therefore, may be a peripheral phenomenon.

Table II shows glycosaminoglycans distribution data (as percentage of total) for zero-time tissues and two groups of implants: (i) 14–44 days and (ii) 66–179 days. These data indicate that the older implants (66–179 days) contain significantly decreased amounts of chondroitin-4 sulfate compared with the original non-implanted tissue and to the younger implants. However, the difference between the older implants and normal bands was not significant. The hyaluronic acid of the implants increased relative to the time of implantation, but was significantly lower in all Dupuytren's tissue implants when compared with normal tissue. Dermatan sulfate levels did not change significantly with time of implantation and was not significantly different from the control normal tissue at any time.

Discussion

Implants of Dupuytren's contracture tissue into the athymic nude mouse do not exhibit histologic signs of rejection and the histologic character of the implant remains essentially unchanged even up to the longest time studied of 179 days. However, the size of the implants and the glycosaminoglycans distribution changes with time. The reduction in volume of the DC

implants is not unique to this lesion. A similar reduction was found in the study of implants from hypertrophic scar and keloid (12). In the case of hypertrophic scar, the implants were reduced to 50% of the original size by 115 days, whereas keloids were reduced to the same percentage by approximately 67 days. As the size of the DC implants (measured by volume) decreases with time without major changes in morphologic character of the implant, the loss apparently affects only the periphery of the implants. The histologic studies used in this report involve largely the structure of mature collagen. Collagen on the periphery could be removed without affecting the structure of the center. This is in agreement with the observation that the implant displays rounded corners and becomes ellipsoid in shape. In some cases a thin layer of the outermost collagen fibers of the implant also appears to reorient parallel to the long axis of the implant. Immediately covering the implant is the subcutaneous loose connective tissue of the mouse. This layer, along with the vascular growth from the mouse, which arborizes through the peripheral most layer of the implant constitute a kind of "capsule."

Microvascular anastomoses are established within approximately 2 weeks after implantation. The extent of the anastomosis is a function of the vascular condition of the tissue prior to implantation since no microvessels are added or appear to be lost. Furthermore, the occluded microvessels present at zero time do not become patent in the implants.

The distribution of glycosaminoglycans in Dupuytren's contracture tissue is similar to those found in other conditions of proliferating connective tissue, i.e, active keloids, hypertrophic scars (13), granulation tissue (14), and healing myocardial infarction (15). All of these conditions exhibit increased chondroitin 4-sulfate when compared with that of corresponding normal tissue. Our data confirm the report of Flint *et al.* (9) who reported increased chondroitin sulfate levels in Dupuytren's tissue. The decrease of chondroitin 4-sulfate in the implants with time in this study is in contrast to the lack of changes in the morphologic character, although further studies using electron microscopy may be useful.

Similar decreases, but of a slower rate, in chondroitin 4-sulfate have also been noted in our studies of hypertrophic scar and keloid implants into nude mice (11, 12). These changes in the glycosaminoglycans distribution in implanted tissue may indicate a missing factor present in individuals with Dupuytren's contracture, or that the nude mouse still has some ability to reject foreign tissues. The increase of hyaluronic acid and decrease of chondroitin 4-sulfate suggest a return of the tissue toward resembling normal palmar fascia. However, the increase of dermatan sulfate, although not statistically significant, suggests conversion to mature scar tissue. These changes make more difficult the use of the implants as a proposed model for evaluation of therapy. However, known procedures affecting the rate of change would be observed well within the time frame of maintenance of the implant.

Dupuytren's contracture is typically treated by sur-

Table I. Volume of Implants at Various Times after Implantation

Days after implantation	No. of mice	No. of implants[a]	Average volume of measured implants (length × width × thickness) mm^3
0		—	126.7
14–27	4	8	114.0
36–69	6[b]	14	85.1
104–109	4[c]	10	43.1
127–136	2	4	36.0
179	1	2	7.3

Regression analysis for volume × time of implants

No.	Slope	Significance	Intercept
27	−0.70152	0.0141	126.7

Slope = change in volume % per day

[a] Eleven of the implants were used for other procedures and not measured.
[b] Four mice received two implants each, two mice received three implants each.
[c] Two mice received two implants, two mice received three implants each.

Table II. Average Clycosaminoglycans Distribution in Dupuytren's Contracture Tissue before and after Implantation

	No. of samples	% of total glycosaminoglycans		
		Hyaluronic acid	Dermatan sulfate	Chondroitin-4 sulfate
Before implantation	6	22.4[a] ± 2.4[b]	54.3 ± 2.0	23.3[a] ± 2.7
Implants 14–44 days	6	26.03[a] ± 1.1	57.5 ± 1.8	16.5[a] ± 1.2
Implants 66–179 days	7	32.5[a, c, d] ± 1.5	59.5 ± 2.5	8.0[c, d] ± 1.5
Normal fascial bands	3	44.3 ± 1.2	50.2 ± 1.1	5.5 ± 0.9

[a] Significantly different from the normal fascial bands at the 1% level of significance.
[b] Values are mean ± SEM.
[c] Significantly different from original Dupuytren's tissue at the 1% level of significance.
[d] Significantly different from the 14- to 44-day group at the 1% level of significance. Student's *t* test was used for the analysis of variance.

gical intervention; recurrence is apparently a significant problem. Hueston states recurrence is more likely if the local skin is left in place after fasciotomy (3). However, MacCallum and Hueston (16) state that perivascular proliferation may be centrally involved in initiating the lesion. By inference, this may be involved in recurrence. Because the lesion appears to retain its morphologic character as an implant, we believe it constitutes a model for studies of growth and maintenance of DC and for therapeutic modalities applied toward conservative resolution.

This research was supported, in part, by NIH Research Grant 5R01GM34928. We extend our sincere appreciation to Michael Jaqua and Peggy Krasovich for valuable technical assistance. We are deeply indebted to Edwin D. Savlov, M.D., Amarillo, Texas, for provision of the Dupuytren's and normal tissue.

1. McGrouther DA. The microanatomy of Dupuytren's contracture. Hand **14**:215–236, 1982.
2. VandeBerg JS, Rudolph R, Gelberman R, Woodward MR. Ultrastructural relationship of skin to nodule and cord in Dupuytren's contracture. Plast Reconstr Surg **69**:835–844, 1982.
3. Hueston JT, Tubiana R, Ed. Dupuytren's Disease. 2nd ed. New York: Grune & Stratton, 1974.
4. Krebs H. Erahrungen bei 350 operative behandelten Dupuytrenschen kontrakturen. Langenbecks Arch Klin Chir **338**:67–80, 1975.
5. Hueston JT. In Converse JM, editor: The hand and upper extremity. Philadelphia: WB Saunders, vol **6**: pp3403–27, 1977.
6. Hueston JT, Hurley JV, Whittingham S. The contracting fibroblast as a clue to Dupuytren's contracture. Hand **8**:10–21, 1976.
7. Kischer CW, Speer DP. Microvascular changes in Dupuytren's contracture. J Hand Surg **9A**:58–62, 1984.
8. Bazin S, LeLous M, Duance VG, Sims TS, Bailey AJ, Gabbiani G, D'Andiran G, Pizzalato G, Browski A, Nicoletis C, Delaunay A. Biochemistry and histology of the connective tissue of Dupuytren's disease lesions. Eur J Clin Invest **10**:9–16, 1980.
9. Flint MH, Gillard GC, Reilley HC. The glycosaminoglycans of Dupuytren's disease. Connect Tissue Res **9**:173–179, 1982.
10. Larsen RD, Takagishi N, Pasch JL. The pathogenesis of Dupuytren's contracture. J Bone Joint Surg [Am] **42**:993–1007, 1960.
11. Shetlar MR, Shetlar CL, Hendricks L, Kischer CW. The use of athymic nude mice for the study of human keloids. Proc Soc Exp Biol Med **179**:549–552, 1985.
12. Kischer CW, Pindur J, Shetlar MR, Shetlar CL. Implants of hypertrophic scars and keloids into the nude (athymic) mouse: Viability and morphology. J Trauma (in press).
13. Shetlar MR, Shetlar CL, Chien S-F, Linares HA, Dobrkovsky M, Larson DL. The hypertrophic scar. Hexosamine containing components of burn scars. Proc Soc Exp Biol Med **139**:544–547, 1972.
14. Shetlar MR, Shetlar CL, Kischer CW. Glycosaminoglycans in granulation tissue and hypertrophic scars. Burns **8**:27–31, 1981.
15. Shetlar MR, Shetlar CL, Kischer CW. Healing of myocardial infarction in animal models. Tex Rep Biol Med **39**:337–338, 1979.
16. MacCallum P, Hueston JT. The pathology of Dupuytren's contracture. Aust N Z J Surg **3**:241–250, 1962.