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原　　著

Experimental Study on Tendon Transplantation Using Allograft

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Injury to the tendon is one of the commonest injury at the daily practice of orthopaedic surgeons. Repair of the large defect of the tendon has been a difficult problem and various methods have been employed during past two decades.

The authors have been developing the method to produce tendon allograft using allogenic tendon which is mainly composed of collagenous tissue. Collagen is the principal component of tendon tissue. However, antigenicity exists in cells of the tendon tissue, and there is minimal antigenicity in collagen itself. It is considered that most of the antigenicity of collagen exists in telopeptide; therefore, if telopeptide is removed, much less antigenicity will be produced, either in an allograft or xenograft of collagen, and there will be no further clinical problem.

In these experiments, the authors tried to produce and preserve the allograft chemically to suppress antigenicity, and grafted it in the defect of rabbits' tendon. The following observations were done: (1) comparison study of tendon allografts treated with various degree of cross linkage of collagen, (2) histological and biomechanical study of the bone insertion area which is one of the weakest point in the graft.

Experimental Methods

(1) Preparation of tendon.

Flexor digitorum profundus tendons were obtained from the frontal paw of adult rabbit donors and treated as follows: extraction of lipid substance was done with 100% ethanol (24 hours), 100% ether, 100% ethanol (1 : 1, 24 hours), 100% ether (24 hours), 100% ethanol (24

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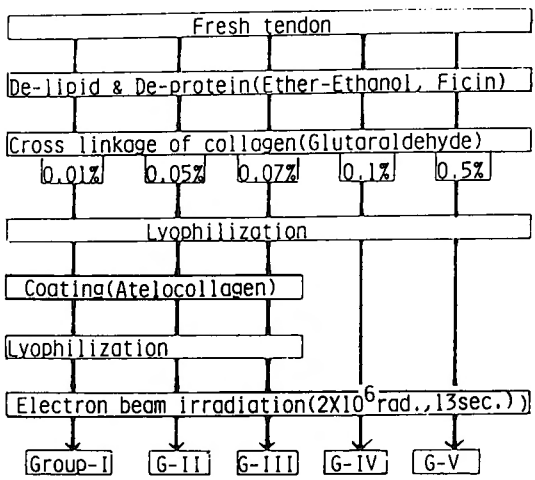

Fig. 1. Processing of tendon for the allograft. According to various processing methods, rabbits were divided into five groups (Group I-V).

hours). After irrigation, extraction of protein substance was done by using 0.05% ficin (pH 7, 24 hours). The tendon were divided into five groups (I–V), using glutaraldehyde for developing cross linkage of collagen in concentrations of 0.01% (Group I), 0.05% (Group II), 0.07% (Group III), 0.1% (Group IV), and 0.5% (Group V) (pH 5, 30 minutes). (Fig. 1) After irrigation, coating with atelocollagen (0.1%, pH 3, 48 hours) was done in Group I, II and III. All of the five groups were irrigated in buffer, lyophilized, irradiated with two million rad. (13 seconds) for sterilization, and packaged for use.

(2) Comparison study of tendon allografts treated with various degree of cross linkage of collagen:

An excision of the third flexor digitorum profundus in the frontal paw of each of eighty-five adult rabbits was operated on and replaced with the preserved allograft of the various groups. In addition, transplantation of a fresh autograft and a fresh allograft was done as control. Produced defect was ca. 3 cm in length. At a proximal aspect and using a microscope, the graft was sutured to the donor's stump by a double right angle method, and sutured to the digit in the peripheral area with a pull-out method. The rabbit groups were killed 2, 4, 6, 8 and 12 weeks after transplantation, respectively. The tendon was excised and fixed with 10% formalin. Histological examination was done with H.E. and Azan stainings.

(3) Study on bone insertion of tendon allograft:

Thirty rabbits each weighing approximately 2.5 kg were used. Paramedian longitudinal skin incision was performed on the base of the third phalanx. The tendon sheath was incised vertically, and with the pulley left remaining, the deep toe flexor tendons were resected 3 cm from the tendon insertion. A processed tendon allograft was grafted at this defect. The proximal region was sutured according to the Kessler method. At the distal region, a hole was drilled into the phalanx. The tip of the tendon is then led into the hole using the simple pull-out

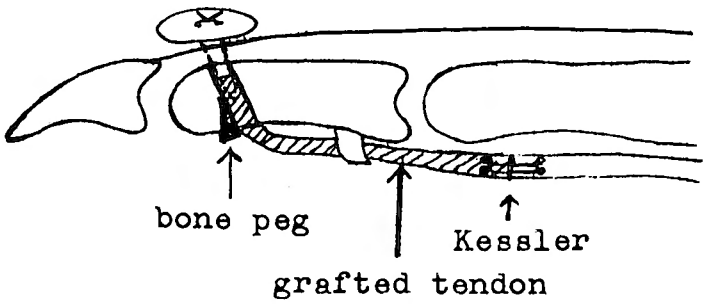

Fig. 2. Method of grafting.

method, and bone fragment is inserted there (Figure 2). For the control, after resecting the tendon of the fourth phalanx, a fresh autologous graft was performed. Postoperatively, cylinder cast was applied for fixation for 4 weeks. Histological study and biomechanical test with Schopper's textile tensile strength tester were done, at 3, 6 and 12 weeks postoperatively.

Results

(1) Effect of various degree of cross linkage of collagen on tendon allograft.

Rupture of the graft at the middle area was observed in Group I. It was considered that due to the low concentration of glutaraldehyde, the graft tissue was softened. No adhesion occurred with the surrounding tissue at the junction. But neoformation of capillary, proliferation of fibroblast and histiocyte in the center area of the graft, and round cell infiltration surrounding the suture could be markedly observed. Fibroblast infiltration was also observed surrounding the graft (Fig. 3).

A slight gap at the junction was noticed in Group II. The graft remained but with minimum adhesion with the surrounding tissue. There were marked proliferations of fibroblast at the junction. At four weeks, both fibroblast infiltration in the middle of the graft and regeneration of fibrous tissue were observed. In the group which was killed at six weeks, regeneration of collagen fibers was observed throughout the graft. These regenerated fibers were arranged parallel to the long axis of the tendon in tortuous fashion. The allograft was mostly replaced by host tissue with numerous proliferations of fibroblast at eight weeks (Fig. 4-a, b). At twelve weeks, collagen fibers were arranged longitudinally and showed a tendinous appearance.

In Group III, in comparison with Group II, a little higher concentration of glutaraldehyde was used for cross linkage of collagen, and no altered appearance was observed. After transplantation, no gap was formed and no rupture at the center area was realized. But the fibroblast infiltration to the middle part of the graft was delayed at six weeks (Fig. 5). At eight weeks, the replacement of fibroblast and regeneration of collagen fibers revealed the same appearance as Group II.

The tissue of Group IV was stiffer than Groups II and III. A separation at the junction was observed and a gap with transparent tissue was present. At four weeks, the fibroblast in-

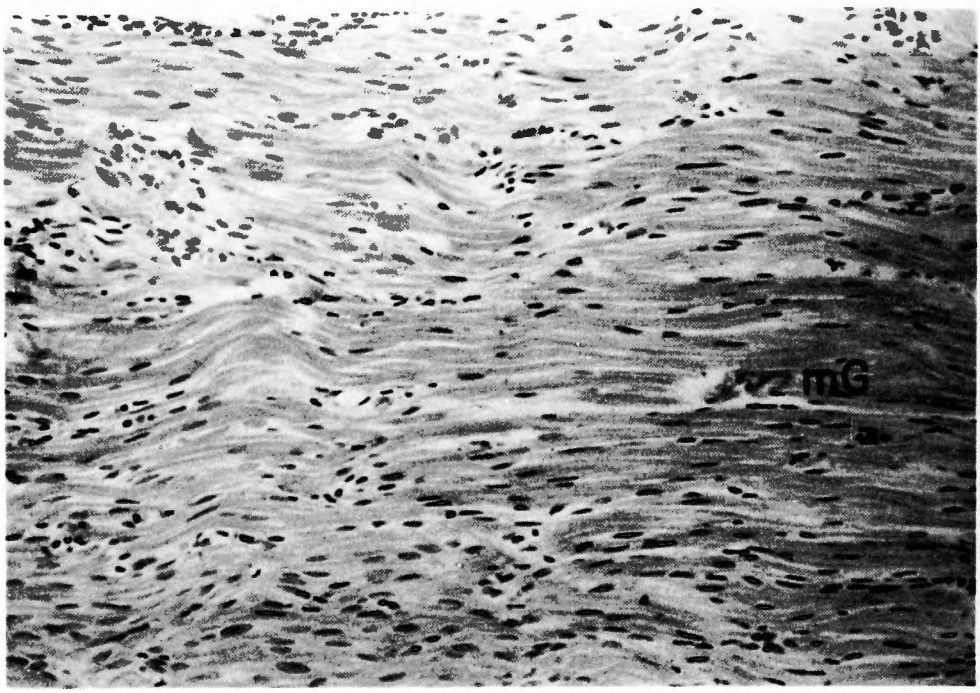

Fig. 3. Group I. Fibroblast infiltration with scattered round cells is observed in the center of area of the graft. (4 weeks postoperatively, Haematoxylin and eosin, ×100) (mG: middle area of the graft)

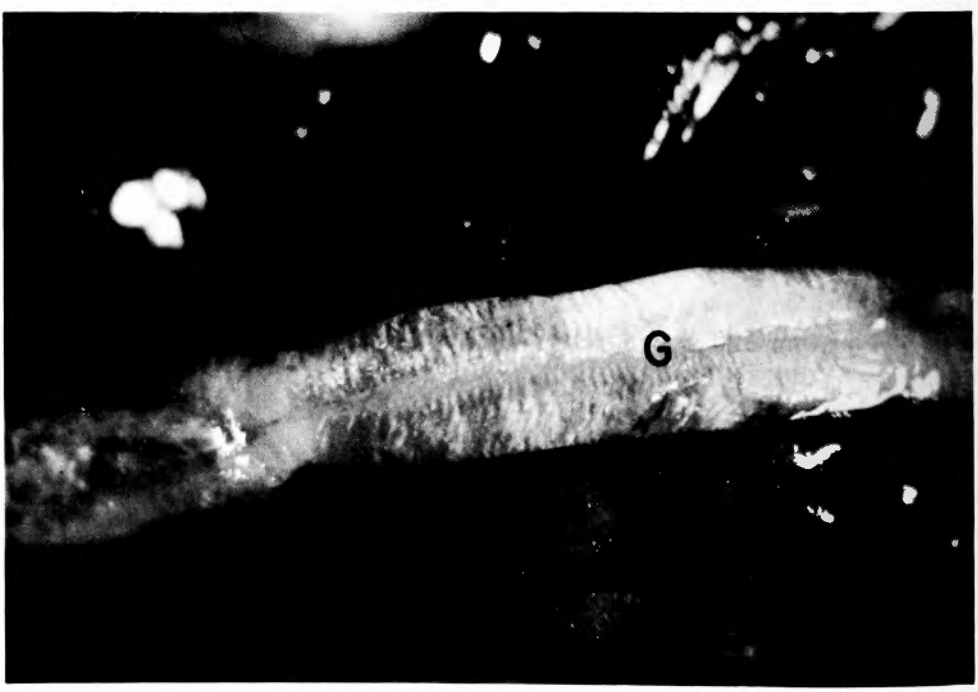

Fig. 4-a. Group II. The graft remains with minimal adhesion. (G: graft) (12 weeks postoperatively)

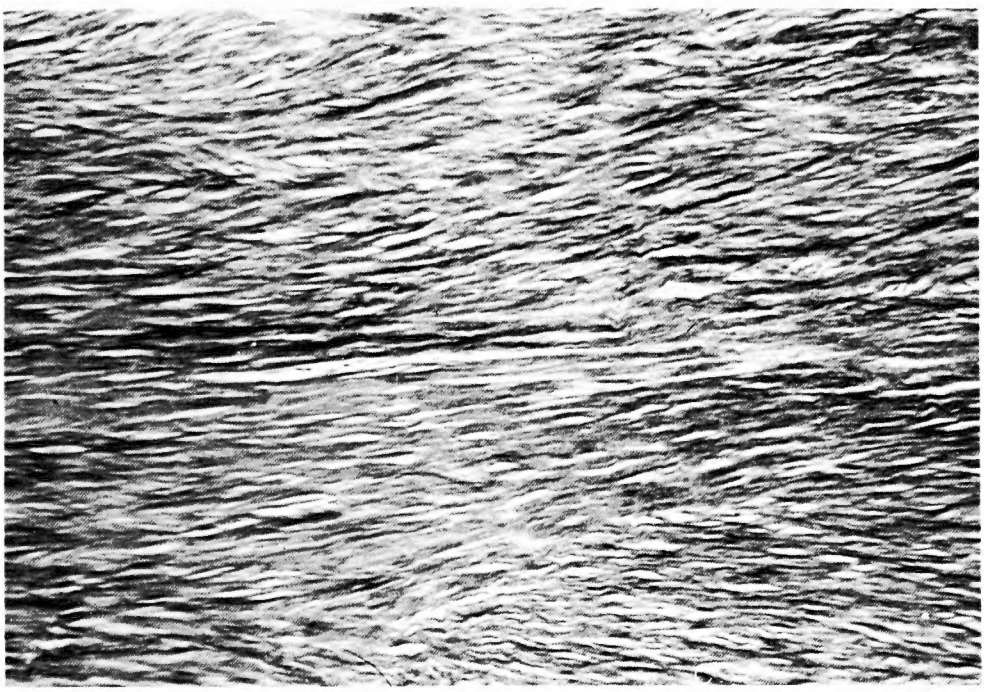

Fig. 4-b. Group II. Regenerated collagen fibers are arranged longitudinally and show a tendinous appearence in the graft. (12 weeks postoperatively, Azan, ×100)

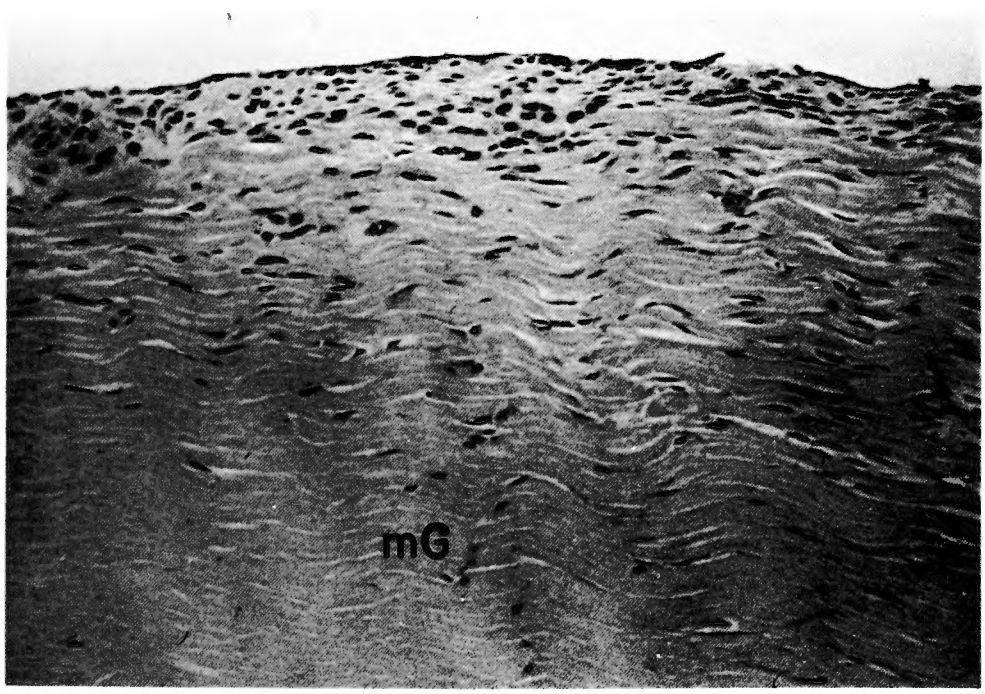

Fig. 5. Group III. Fibroblast infiltration is observed surrounding the graft, however, which is delayed in the middle part of the graft. (16 weeks postoperatively, Haematoxylin and eosin, ×100) (mG: middle of the graft)

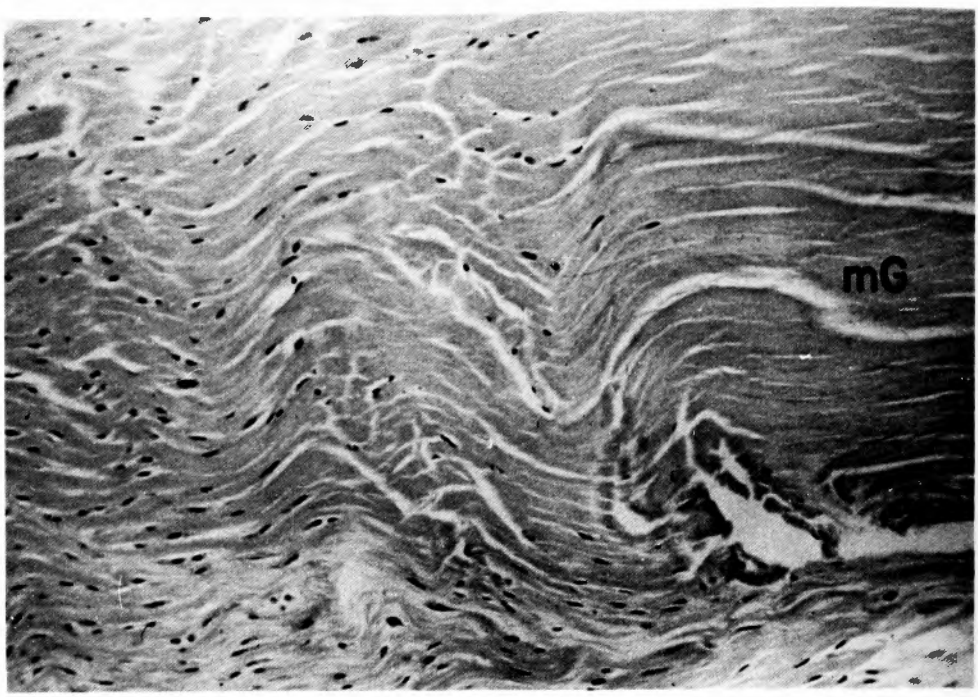

Fig. 6. Group IV. Fibroblast infiltration is rarely seen in the middle of the graft with homogenous appearance. (8 weeks postoperatively, Haematoxylin and eosin, ×100) (mG: middle of the graft)

filtration in the middle area of the graft was observed. However, the cell component was rarely seen in the center of the implant even at eight weeks (Fig. 6). Regeneration of collagen fibers was revealed at twelve weeks, but with much less degree of regeneration than with Groups II and III.

In Group V, due to the concentration of glutaraldehyde which was five times that of Group IV, the tissue was very stiff. No infiltration of fibroblast was observed and at twelve weeks the existing graft showed an invested appearance (Fig. 7). At twenty-four weeks, it still had the same appearance and no tendinous tissue replacement was seen.

With the fresh tendon autograft, fibroblast infiltration was noticed at two weeks. It extended from the stump and surrounding tissue to the middle area of the graft (Fig. 8). Regeneration of tendinous tissue was seen at six weeks. At two weeks after fresh allografting, the disappearance of the nuclei of cells in the graft was partly observed. At four weeks, fibroblast infiltration in the graft was markedly noticed (Fig. 9). The fibroblast reached the center of the implant at six weeks and regeneration of the collagen was well developed.

(2) Study on bone insertion of tendon allograft.

Histological examination revealed no obvious difference at the bone insertion of the tendon between autograft and allograft, at 3 weeks postoperatively. There were marked round cell infiltration at the contact area with bone. Fibroblast infiltration was observed at proximal suture

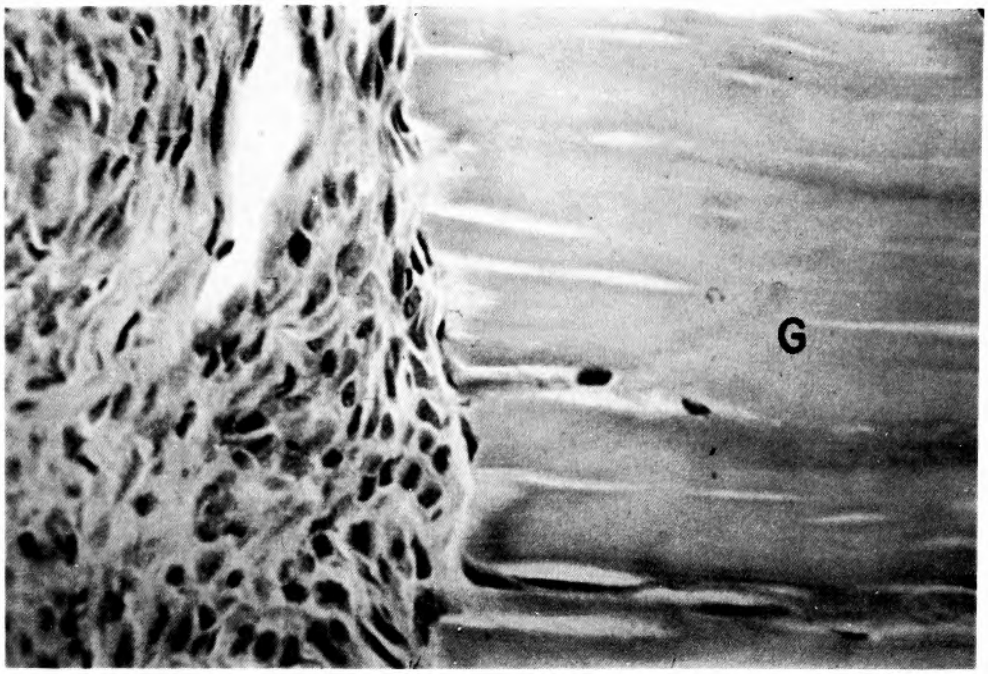

Fig. 7. Group V. No infiltration of fibroblast is observed in the graft. Round cell infiltrates around the graft. (12 weeks postoperatively, Haematoxylin and eosin, ×400) (G: graft)

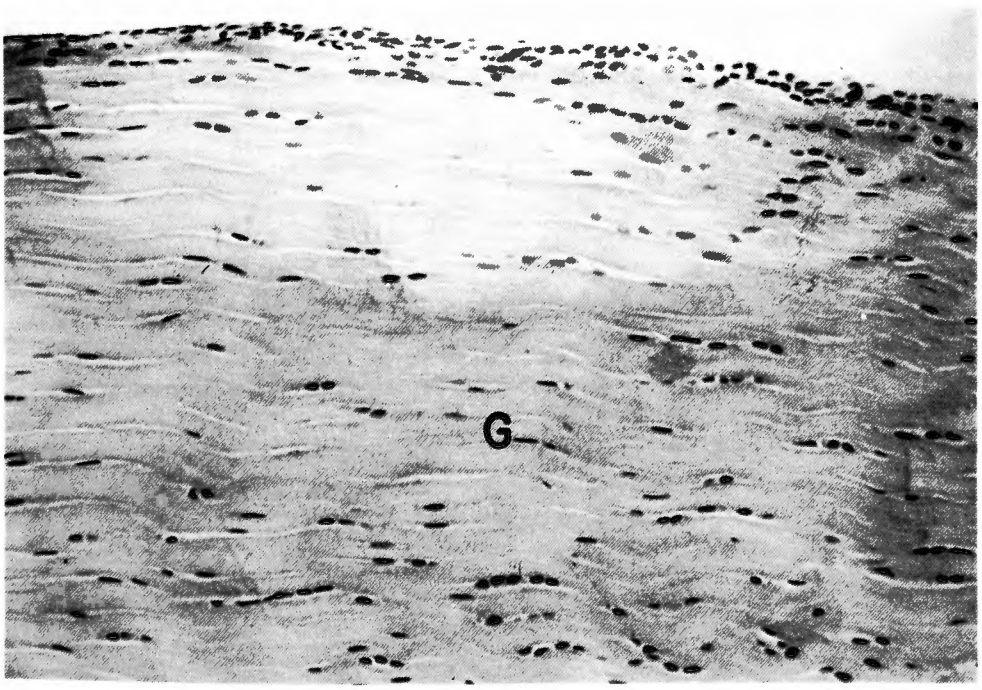

Fig. 8. Fresh autograft. Fnfiltration of fibroblast is seen in the middle area of the graft. (4 weeks postoperatively, Haematoxylin and eosin, ×100) (G: autograft)

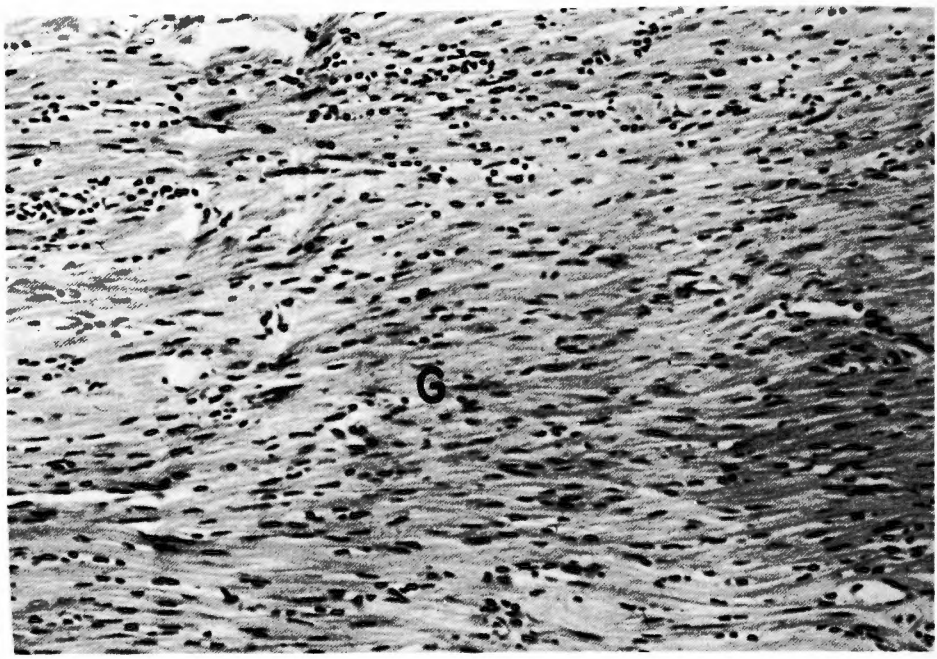

Fig. 9. Fresh allograft. Moderate fibroblast infiltration with round cell invasion is noticed in the graft. (Haematoxylin and eosin, ×100) (G: allograft).

and distal side of the graft, developing into the middle graft. However, there was no fibroblast infiltration at the central region of the graft, where tortuosity and partial ruputure of collagen

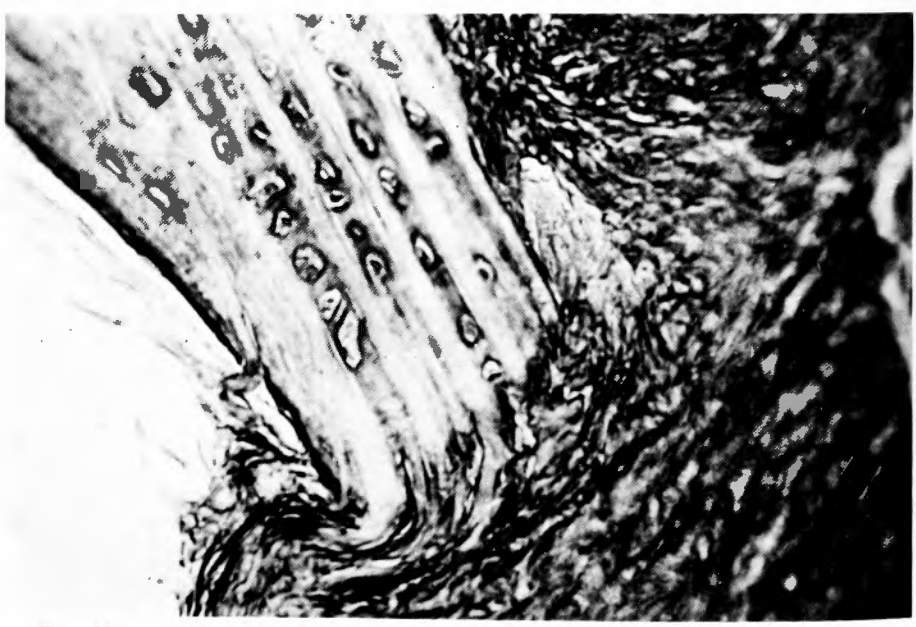

Fig. 10. 12 weeks after grafting the preserved tendon, collagen fibers are seen permeating into the bone. (Azan Mallory, ×200)

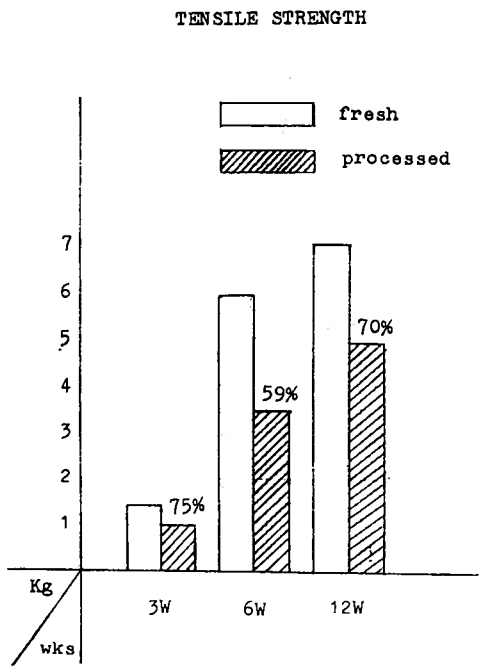

Fig. 11. Result of tension experiment: Percentage (%) corresponds with the strength of the preserved tendon compared to the fresh autologous tendon.

fibers were observed. At 6 weeks, gap between bone and tendon is filled up with infiltration of connective tissue, but showed no lamellar structure as seen in normal tendon insertion to the bone. Abundant fibroblasts are seen at the area in allograft, but minimal in autograft. At 12 weeks, preserved (processed) allograft showed decrease of number of fibroblast and progressive invasion of collagen fibers into bone with much less tortuosity, as seen in fresh autograft. (Figure 10).

Biomechanical study revealed the following results. For the tension experiment, a textile tensile strength tester (Schopper) with an improved zipper was used. In the control fresh autograft, the distal end can be pulled out from the bone using an average of 1.4 kg of force at 3 weeks following surgery. With the preserved allograft, by contrast, 75% of the power of autograft is required to pull out from the bone. Six weeks after surgery in the fresh aurtograft, avulsion from the contact region with the bone occurs with an average of 5.9 kg. Rupture of the allograft occurs at the central region of the graft with 59% of the power of the autograft. Twelve weeks following surgery, both groups display increased strength of the insertion, and avulsion from the contact region with the bone. The strength of the preserved allograft increases to 70% of that the autograft (Figure 11).

Discussion

For the repair of large defect of tendon, autograft has been used clinically with satisfactory results. However, autograft has some disadvantages as follows: (1) scar and pain at the donor site, (2) extensive operative invasion to obtain grafts, (3) prolonged operative time, (4) func-

tional disturbance by obtaining multiple tendon grafts. To improve the results of treatment of severe and multiple injury of tendon, tendon allograft should be developed in future. There are also many problems in tendon allografting such as (1) immunological and foreign body reaction, (2) tensile strength, (3) tissue affinity with host tendon, (4) adhesion, (5) preservation method, (6) sterilization method, and (7) choice of donor and morality of transplantation.

Since REHN[9] first reported the method of using other materials instead of autograft, many trials have been carried out to improve the unsatisfactory points above. TENEFF[10] and GRAHAM[4] investigated frozen-stored tendon; ISELIN[6] and CORDREY[2] used Cialit, merthiolate and alcohol treated graft; Cordrey *et al.*, PPTENZA[8] and CAMESON[1] *et al.* used lyophilization method. They reported some regeneration of the tendinous tissue. In these investigations, transplantation of the preserved allograft demonstrated a more delayed healing process than did the tendon autograft. However, the replacement and the reformation of the tendon were observed even in the allograft. PEACOCK[7] and FLYNN[3] protest that the allograft is basically similar to implantation of a bundle of collagen fibers. The antigenicity of the collagen fiber has been realized, but has rarely showed clinical problems. Nevertheless, in allograft it is necessary to recognize the existence of antigenic components other than collagen.

The purpose of this experiment was to bridge the large defect of tendon allograft which is mainly composed of a frame of collagen fibers but is without a cell component. The authors investigated allograft of the peripheral nerve previously. According to the results of the experiment, use of ether and ficin for suppression of antigenicity were suggested, as well as lyophilization for tissue preservation, and electron beam irradiation for sterilization[5]. The same methods were applied in this experiment, and glutaraldehyde was also used to moderate tensile strength and achieve good tissue affinity.

Using our methods of immersing the treated (processed) tendon into normal saline resulted in a slight expansion with a white glossy surface. When compared with fresh tendon tissue, this was a little stiffer but had no alteration in appearance. Histologic observation appeared as a frame of collagen fibers in lack of a cell component. However, the higher the concentration of glutaraldehyde used and the longer the time treated, the stiffer the graft that was obtained. The graft treated with 0.1% glutaraldehyde was very hard and difficult for cell infiltration, whereas in that treated by only low concentrations of glutaraldehyde for a short time the tissue ruptured easily. Accordingly, we had the best results in Groups II and III, where good cell infiltrations from the host were observed and appropriate tensile strength was obtained. By comparison, there were satisfactory findings in Group II, in which a good tissue affinity and a fixed union of sutured sites were observed. Macroscopical and histological comparison with autograft is shown in Table I. Results of preserved tendon allografting in this experiment showed us the usefulness of: (1) ether and ficin for extraction of lipid and protein to suppress antigenicity; (2) glutaraldehyde in appropriate concentrations for moderate tensile strength and good tissue affinity; (3) atelocollagen coating for cell guidance to graft; (4) lyophilization for storage; (5) cathode irradiation for tendon sterilization.

According to the biomechanical tension test, there are no rupture at the proximal suture nor

Table 1. Findings of allograft and autograft.

Findings \ Group	I	II	III	IV	V	Autograft
Rupture	+	−	−	−	−	−
Union of Sutured Site	++	+	+	±	−	++
Adhesion	±	±	±	±	±	±
Round Cell Infiltration around Graft	±	±	±	+	++	±
Fibroblast Infiltration in Graft	++	++	+	±	−	+
Parallel Arrangement of Collagen	−	+	+	±	−	++
Replacement of Graft by Tendinous Tissue	−	+	+	±	−	/

at the tendon center, and just as in the autologous tendon, the portion inserting into the bone is pulled out at 3 weeks. At 6 weeks following surgery, the preserved tendon and autologous tendon display differing conditions. While there is avulsion at the insertion with the autograft, there a tendon rupture in the central region of graft with the preserved allograft (Figure 12).

Concerning strength of preserved allograft, its condition prior to and immediately following surgery equals that of autograft. However, from 4 to 6 weeks after grafting, allograft start to lose the strength, during regeneration of collagen fibers of the host with softening of original

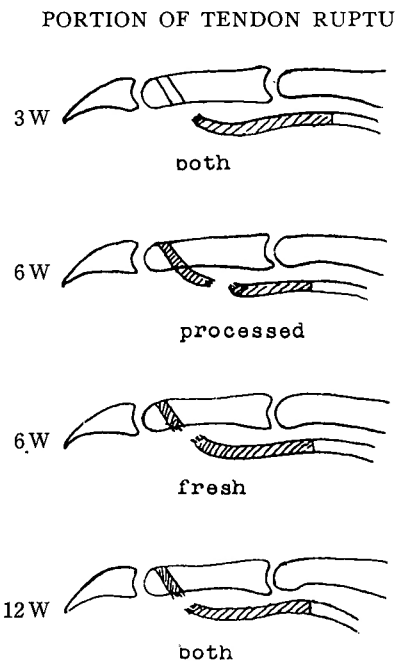

Fig. 12. Figures show the disconnected area during the tension experiment. At the third week, the tendon insertion (attachment) can be pulled out in both groups. At the sixth week, disconnection occurs in the central region of the graft in the preserved tendon. Meanwhile, rupture at the tendon insertion (attachment) occurs with the autologous tendon. By the twelfth week, both groups show rapture at insertions, but not at the proximal graft.

collagen fibers of graft. Strength at this stage is inferior to that of a autograft, but by the 12th week following surgery, the preserved tendon gains a strength approximately 70% of that of the autologous tendon insertion. Ruptures occur at insertion, but not in the middle of the graft in both groups. We believe our preserved allograft can be of use for injuries involving sereral fingers where an autograft alone would not be effective.

Sammary

(I) Comparison study of tendon allografts treated with various degrees of cross linkage of collagen.

1) All groups of preserved tendon revealed minimal adhesion, and no resorption of the graft by inflammation was observed.
2) Early rupture of graft at the middle area was observed in Group I.
3) In Group II, no separation or rupture was seen. At twelve weeks, the replacement of tendinous tissue had developed.
4) In Group III, rupture and separation were rare but, compared with Group II, there was some delay in the repair of the tissue.
5) In Group IV, there was a separation at the junction and the repair was delayed.
6) The preserved graft was hard in Group V and repair of tissue was not seen.
7) Accordingly, the preserved allograft in Group II is considered to be effective for clinical employment.

(II) Study on bone insertion of tendon allograft.

1) A tendon graft was performed using a preserved allograft (Group II) we synthesized, and investigation was done mainly on the distal tendon insertion.
2) Three weeks following surgery, no disconnections occured at the proximal suture, and it was possible to pull out the distal tip from the bone.
3) Six weeks following surgery, rupture occurs in the central region of the preserved tendon.
4) Twelve weeks following surgery, avulsion from the bone occurs at the tendon insertion, and the preserved allograft gains a strength 70% of that of the autograft.
5) Our preserved allograft can be applicable for high degree injuries which the autograft alone cannot handle. As the strength of the tendon decreases between the 4th and 6th weeks, post-operative caution is imperative.

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和文抄録

同種腱移植に関する実験的研究

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成熟家兎の深指屈筋腱を採取し，100％エーテル，100％エタノールで脱脂，0.01％フィシンで脱蛋白後，グルタールアルデヒドで架橋した．グルタールアルデヒドの濃度により5群に分けた。0.01％をⅠ群，0.05％をⅡ群，0.07％をⅢ群，0.1％をⅣ群，0.5％をⅤ群とした．これを凍結乾燥し，0.1％アテロコラーゲンでコーティングした後，再び凍結乾燥を行った。こうして保存したものを使用前に殺菌と免疫反応を抑制する意味で電子線を照射して使用した．

成熟家兎の第3指に対し，pulley を残して腱鞘，浅深指屈筋腱を切除し，深指屈筋腱に処理腱を pulley 内に通して移植した．移植片の中枢側は double right angle 法を応用し埋没縫合し，末梢側は pull out 法にて指骨に埋没した．移植後はギプス固定を施した．処置後2，4，8，12週で屠殺して組織学的検索を行った．各群とも移植片と周囲との癒着は少なく，中枢縫合部で軽度の癒着を認めるが，滑動性に問題はなかった．

Ⅰ群はグルタールアルデヒド架橋が弱く，全例に移植片中央部で断裂を認めた．Ⅱ群は2週では中枢縫合部で毛細血管の新生と線維芽細胞および組織球の増殖がみられた．4週では線維芽細胞が主に縫合部から移植片中央部にまで侵入し，6週で移植片全体に膠原線維の再生がみられた．8週では移植片はほとんど置換され，12週では線維束は細いが長軸に沿って膠原線維が並び，ほぼ腱組織の構造を成していた．Ⅲ群はⅡ群よりグルタールアルデヒド架橋がやや強く，線維芽細胞の侵入が遅れ，6週でも移植片中央部で線維芽細胞の侵入を認めない部分があった．Ⅳ群はさらに材質が硬く，8週でも移植片中央部は線維芽細胞が少なかった．Ⅴ群はグルタールアルデヒドの架橋が強すぎるためか12週以降でも線維芽細胞の侵入はほとんどなかった．

以上の結果よりⅡ群が最も適当な処理法と考えられた．このⅡ群の処理腱を用いて力学的検討を加えた．移植法は同様に行ったが，末梢側の pull-out 部の腱と骨との間隙に小骨片を挿入した．コントロールとして新鮮自家腱を移植した．強度試験にはショッパー型織物引張試験機のチャック部を改良したものを用いた．術後3週では対照群の自家腱移植では平均 1.4 kg の力で末梢端が骨より引抜けた．処理腱では自家腱の75％の力で骨より引抜けた．術後6週では対照群は平均 5.9 kg で骨との接合部で剝離した．処理腱は自家腱の59％の力で移植片中央部で腱の断裂がおこった．術後12週では両群とも強度を増し骨との接合部で剝離した．処理腱の強度は自家腱の70％にまで回復した．