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TISSUE SURROUNDING GRAFTED TENDONS IN DOGS

A Pathomorphologic Study

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Tendon grafting is a complex problem, in which must be considered a number of factors such as healing at the site of anastomosis, change in morphologic features and in functional properties, influence of surrounding tissue, and adaptation to a change in muscular load.

Reparative or regenerative processes which characterize the healing of a tendon transplant have recently been studied by, among others, Peacock (1965), Flynn & Graham (1965), Birdsell, Tustanoff & Lindsay (1966), and Seiffert (1967). All have found that the reparative pattern is much the same as is seen in different stages of wound-healing. One question which has not yet been answered is how the reaction of the surrounding tissue influences the reparative process in the grafted tendon.

To investigate this question we have carried out a series of experiments, the purpose of which has been to study what occurs in the tissue surrounding a tendon graft and the ultimate fate of a thin tendon graft in different types of surrounding tissue.

MATERIAL AND METHODS

Experiments were carried out in 57 male and female dogs of varying races. The average weight of the dogs was 15 kg. Three of the animals were used for control studies and three for special radioactive investigations. Thus transplantation for morphologic study was performed in 51 dogs. The peroneus longus tendon was used as a graft to replace the Achilles tendon. Parallel to this, however, experiments were designed for comparative reasons in which the peroneus longus tendon was excised and reinserted in its own tendon sheath. In all, the 51 dogs represented 70 transplanting procedures of which 45 were transplants for the Achilles tendons and 25 reimplantations of the peroneus longus tendon.

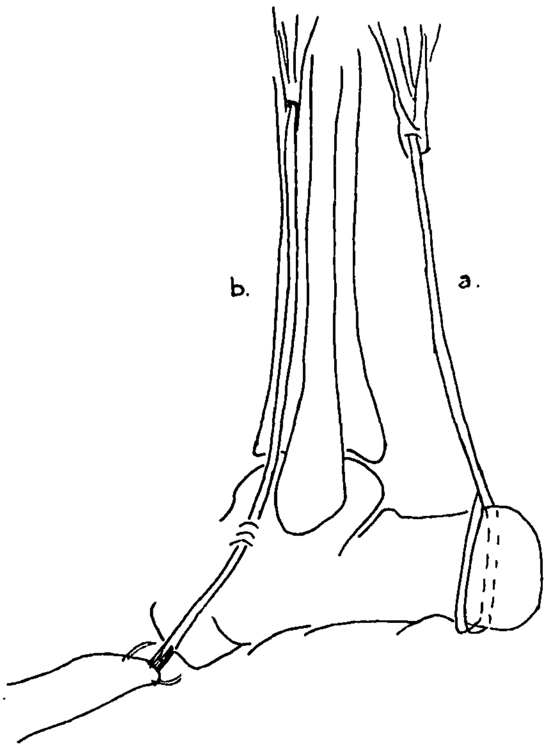

Figure 1.

Figures 1 and 2. Schematic representation of the different surgical procedures (see text).

All operations were performed in general anaesthesia with intravenous administration of Pentothal and a bloodless field under tourniquet was used.

Operative Technique in Transplanting Peroneus Longus Tendon to Substitute Excised Achilles Tendon (a in Figure 1)

The hindleg was surgically prepared and draped. A longitudinal incision on the lateral border of the foot was used to expose the peroneus longus tendon which was then divided. A short curved incision was then made over the lateral aspect of the leg just below the knee joint and the peroneus longus was identified and withdrawn proximally and severed at the musculotendinous junction.

A long curved incision was made on the lateral side of the Achilles tendon from the os calcis up to just below the knee joint. The cutaneous nerves were spared, the whole Achilles tendon was severed at the incised insertion into the os calcis. A drill-hole was made in the os calcis. The Achilles tendon together with its tendon sheath was dissected out and severed proximally at the musculo-tendinous junction.

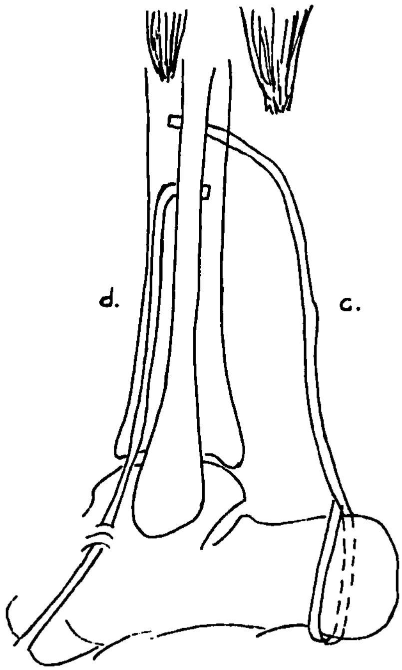

Figure 2.

The peroneus longus tendon was transferred into the prepared bed and united to the musculotendinous junction of the whole Achilles tendon using a pull-through technique. The junction was sutured with Teflon stitches. The tourniquet was removed and the distal end of the Achilles tendon was then pulled through a hole in the os calcis and sutured back to itself thus forming a loop. The load on the tendon was estimated to 350 g. All bleeding spots were carefully tied. The skin was closed with stainless steel sutures and the leg was immobilized in a plaster cast with the knee in full flexion and the foot in semi-flexion.

Operative Technique in Reimplanting the Peroneus Longus Tendon (b in Figure 1)

The hindleg was surgically prepared and draped. The peroneus longus tendon was exposed on the lateral side of the foot and severed. A Teflon suture was attached to the end of the tendon. On the lateral side of the leg a short incision was made just below the knee joint. Through this the proximal portion of the tendon was identified. The tendon was pulled out together with the Teflon thread. The musculotendinous junction was divided obliquely. An elongation suture was made, the whole graft was pulled back in its original place, and an end-to-end suture was made at the distal severance. The skin incisions were closed with stainless steel sutures. The leg was immobilized with a low cast with the foot in semi-flexed position.

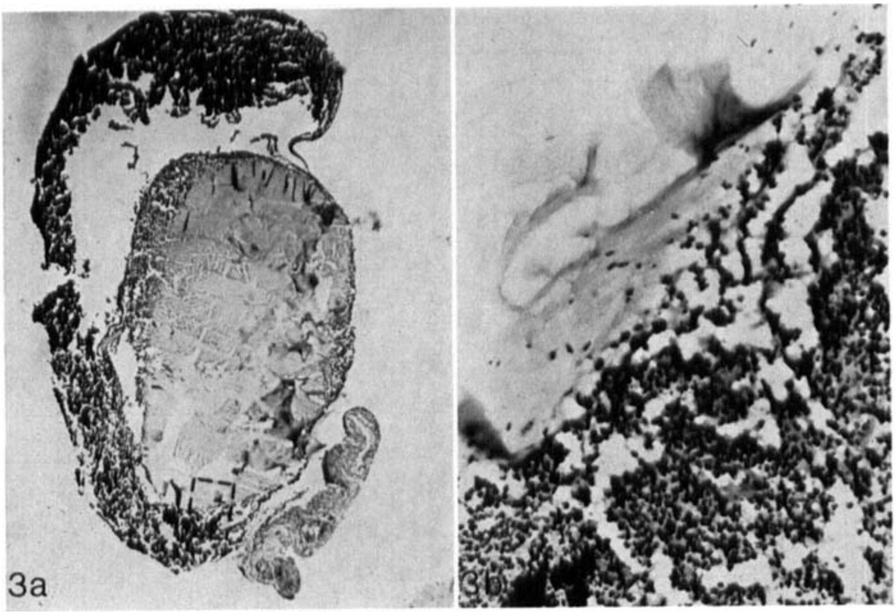

Figure 3 a. Transverse section of transplanted peroneus longus tendon for Achilles tendon in dog. 2 days postoperatively. Verhoeff's connective stain. Extratendinous reaction with abundance of polynuclear leukocytes and small haemorrhages. No abnormalities in grafted tendon.

Figure 3 b. Higher magnification of marked area in 3 a.

"Unloaded" grafts (c and d in Figure 2)

In seventeen instances care was taken that no load be exerted on the grafted tendon. This was achieved by bringing the proximal end of the tendon graft in between the tibia and the fibula to the posterior side of the leg in cases where the peroneus tendon was retransplanted and the anterior side in the cases where it replaced the Achilles tendon.

In all cases excepting those in which the experiment was terminated earlier, the plaster cast remained for some 4 weeks. No animal was left without immobilization. It was possible to keep the dogs from extensive chewing of the plaster by adding a few drops of formaline to the plaster of Paris. The animals were allowed to survive after surgery for various lengths of time (Table 1).

Following the termination of the experiment in each individual case, macroscopic examination and recording were made. Sections were obtained for histologic study, the staining used was haematoxylin and eosin and Verhoeff's connective tissue stain.

RESULTS

As a general statement it can be said that loading either grafted or reimplanted tendons did not seem to alter the structural development

Table 1. Number of legs with different surgical procedure (Figures 1 and 2) and survival days

Days	A	B	C	D	Total
2	3	3	1	1	8
7	5	2	4	2	13
14	3	2			5
21	3	2			5
30	3	2	2	1	8
60	5	3	2	1	11
90	4	2			6
120	4	2	1	1	8
180	4	1	1		6
Total	34	19	11	6	70

of tissue events in and around the tendon. The following description thus applies to both loaded and unloaded tendons.

Transplantation of Peroneus Longus Tendon for the Achilles Tendon

Two days (Figure 3)

Macroscopic: On opening the wound small diffusely scattered haematomas were observed along the transplant. There was abnormal tissue surrounding the grafts and these were slightly swollen. Fibrinous plaques were seen irregularly spread in the grafting area. The suture regions did not disclose anything abnormal.

Microscopic: On transverse sections the tendon proper appeared slightly split up but as there was no sign of oedema or other reaction in these splits, they were regarded as results of the technical handling. The collagenous bundles of the tendon were normal. The tendon was covered by a thin one-cell layer of fibroblasts which was interpreted as the epitelium. Outside of this there was a fibrinous zone which seemed to wall off the tendon from the surrounding tissue. This latter gave the impression of high activity with an abundance of polymorphonuclear leukocytes. Small irregular haemorrhages were observed and occasional strands of erythrocytes which might represent open circulation and might be precursory to the formation of capillaries. Occasional mononuclears were identified.

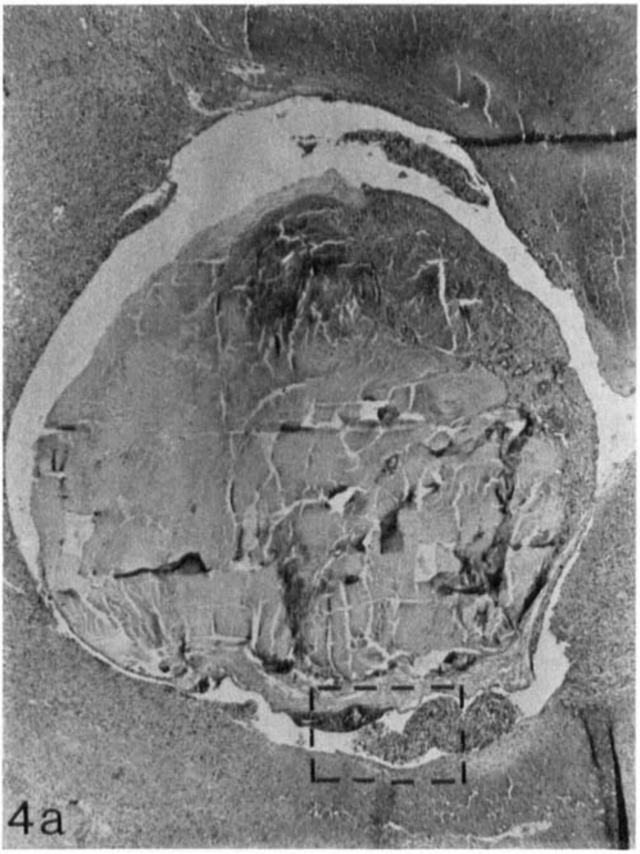

Figure 4 a. Transverse section of transplanted peroneus longus tendon for Achilles tendon in dog. 14 days postoperatively.
Graft surrounded by granulation tissue with tufts growing into tendon. Beginning vascularization of tendon graft. Graft still comparatively intact with almost normal cellularity.

Seven days

Macroscopic: At this time there was a marked difference in the appearance of the tissues surrounding the graft. The tendon was covered by a thin coat of fibrinous substance which, however, seemed enforced by a network of fibrils. This could easily be peeled off the graft. The tendon graft no longer appeared swollen but there was no shiny glittering surface. Instead the surface had a dull yellow colour.

Microscopic: The space around the tendon had diminished but was still there. The thin layer of fibroblasts forming the epitenon around

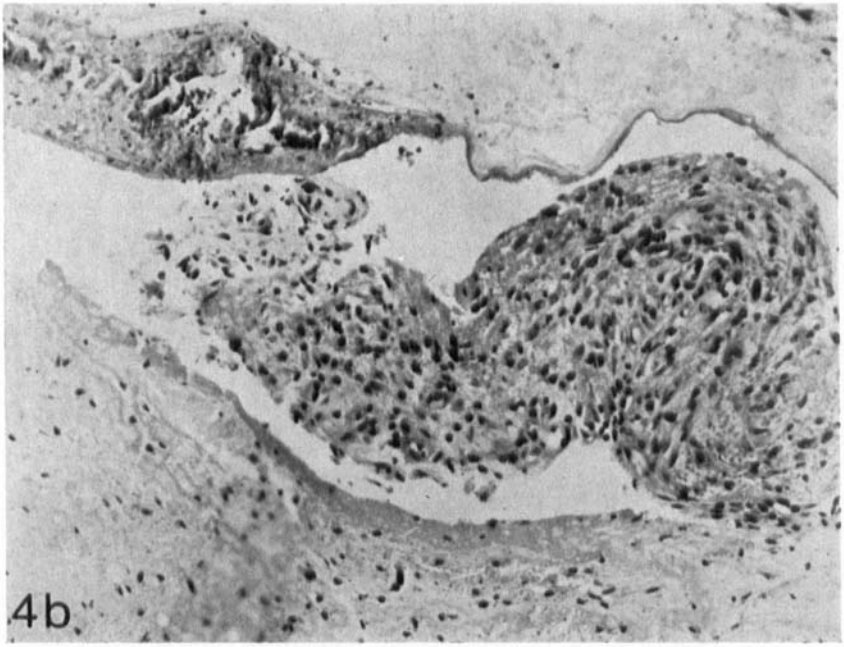

Figure 4 b. Higher magnification of granulation tuft.

the graft still remained intact except for some small regions where vascular connections had been established between tendon and the surrounding tissue which was markedly increased. Occasional capillaries were now noticed to appear irregularly and some of these broke through the epitenon but did not seen to invade the tendon proper. These vessels were surrounded by a number of cells the identification of which was somewhat difficult but which were interpreted as fibrocytes. The same type of cell was seen scattered through the tissue around the tendon. Polymorphonuclear leukocytes were still seen but to a lesser degree. Small clusters of lymphocytes were observed. The surrounding tissue thus resembled true granulation tissue.

Fourteen days (Figure 4)

Macroscopic: The grafted tendon was still surrounded by a coat of tissue which had turned more fibrous but which could still be easily stripped off. This surrounding tissue having become more organized did not occupy such a large area, which gave the impression that the graft was slightly thinner. No oedema was seen and the tendon was slim and slender without any naked-eye abnormalities.

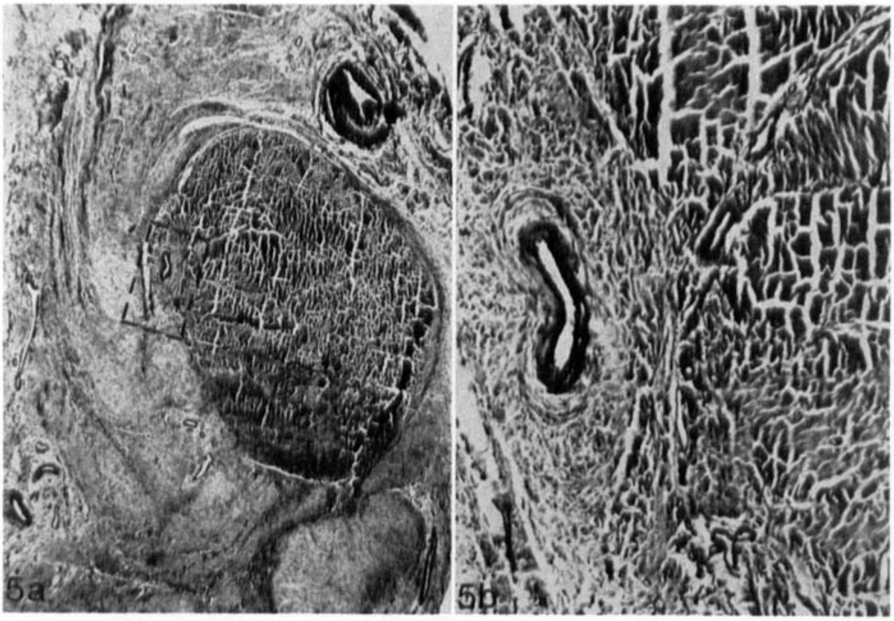

Figure 5 a. Transverse section of transplanted peroneus longus tendon for Achilles tendon in dog. 60 days postoperatively.
Graft intimately associated with surrounding connective tissue, but a border line is still apparent with some vascularization.

Figure 5 b. Higher magnification of marked area in 5 a. Note very intimate association between graft and surrounding tissue with vessels invading graft.

Microscopic: As observed in sections under the microscope the surrounding tissue still appeared abundant but by now fibrous strands were more prevalent and very few polymorphonuclears were seen. There were a great many fibroblasts and some mononuclears. There was no longer any oedema and the space around the tendon had shrunk. The epitenon was still mostly intact but occasional connections were seen between the central parts of the abundant granulation tissue and the tendon. In this, connecting channel-like vessels were seen. The number of capillaries had markedly increased in the tendon.

Twenty-one, thirty, and sixty days (Figure 5)

Macroscopic: The surrounding tissue was now well organized in a comparatively fixed coat which at the end of this observation period was almost white and still could be peeled off from the tendon but with more difficulty as there were adhesions between the surrounding

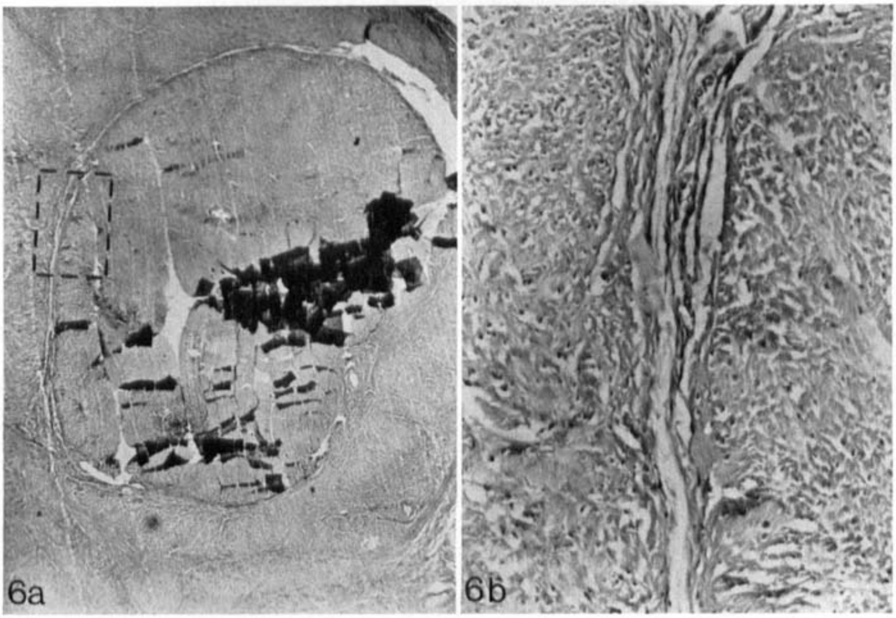

Figure 6 a. Transverse section of transplanted peroneus longus tendon for Achilles tendon in dog. 120 days postoperatively.
Graft morphologically almost identical with surrounding tissue. Border line recognizable as fibrous strand seen in higher magnification in Figure 6 b.

Figure 6 b. Marked area in 6 a of fibrous strand as remnant of border line. Note the almost identical cellularity of graft and surrounding tissue.

tissue and the tendon. The latter had shrunk somewhat and appeared to be split up in places where the adhesions were found.

Microscopic: The granulation tissue had during this time become replaced by fibrous tissue. Fibrous strands reached the epitenon of the grafted tendon and in some places they broke through and invaded the tendon where splits were present. There were no signs of the tendinous strands becoming absorbed by the invading tissue, only pushed aside. The space around the tendon was obliterated almost completely. There was rich vascularity of the fibrous tissue. In the tendon proper, which had diminished considerably, a number of vessels could be seen not only in the periphery but in the centre as well. The collagenous bundles in the grafted tendon had lost their homogeniety and appeared shrunken but structural details were still discernible and no signs of necrosis were noticed. The sutured areas were all characterized by dense fibrous tissue. Polymorphonuclear leukocytes could no longer be seen but small accumulations of lymphocytes and plasma cells were observed.

Ninety-one, one hundred and twenty, and one hundred and eighty days (Figure 6)

Macroscopic: By this time a functional "tendon" was found. The tissue surrounding the tendon graft could hardly be peeled off. The size of the "tendon" corresponded well in all cases to the size of the Achilles tendon of the non-operated leg. It was, however, not as pliable as the original tendinous tissue but served an equally good function as judged by the clinical result.

Microscopic: The original tendon graft had by now become engulfed by the reactive fibrous tissue and only in smaller patches reminiscences of the graft could be seen. The differences between the original graft and the surrounding tissue were almost negligible in most places. Both were highly cellular. Still, however, there was an indication of the original epitenon border which facilitated discovery of the location of the graft in the reactive fibrous tissue. This had become better organized and the fibrous strands were now observed to follow a parallel straight course. The number of vessels had decreased and the overall impression was that the tissue was no longer as active as in earlier stages.

To summarize, it can be said the grafted tendon is replaced by an active reparative process characterized by granulation tissue which eventually turns into fibrous tissue. The original thin graft becomes involved by this process in a homogenous strand of fibrous tissue the size of which corresponds to the tendon it replaces. Microscopically the grafted tendon resists the invading properties of the reactive tissue for a time but finally it succumbs and is replaced by fibrous tissue in which the collagen fibres become organized in a way similar to a tendon. After 180 days there is still a cellularity which supercedes that of an ordinary tendon. Clinically the functional qualities remain unimpaired.

Reimplantation of the Peroneus Longus Tendon in Its Own Sheath

This procedure proved to be quite innoxious to tendon, sheath, and surrounding tissue. For this reason it has not been found of any advantage to give a detailed separate description of the macroscopic and microscopic appearance of these control transplantations.

It can only be said that, apart from a slight reaction with haemorrhage, oedema, polymorphonuclear leukocytes, and slight vascular increase as a response in the earlier stages to the surgical trauma, the development was completely uneventful. No later reactions were observed in either tendon, tendon sheath, or surrounding tissue. Only at the suture levels was there an increase in activity up to about 21 days after surgery. In the mid-portion the tendon graft retained the same size and the same structural characteristics through the control period. No increased cellularity could be observed.

DISCUSSION

In this investigation it has become evident that the morphologic sequence of events differs considerably in tendon grafts surrounded by different types of tissue. The experimental procedures have focused attention on the influence the tendon sheath may have on repair of grafted tendon tissue.

The tissue that surrounds the transplant when it is transplanted without the tendon sheath into loose connective tissue as well as the graft itself demonstrates a morphological development which in time and appearance of various structural elements corresponds to what has been shown earlier by several authors. Adams, already in 1860, stated that in rabbits the tissue that filled an experimental gap ultimately resembled a mature tendon histologically. Conway et al. (1967) excised a portion of the calcaneal tendon in rabbits. The connective tissue growth in their experiment set in rapidly after the tendon had been removed with signs of regeneration as early as after two days, and by 56 days there were connective tissue fibres with characteristics similar to those of mature tendons. By 240 days the regenerated material resembled a mature tendon in almost all qualities except that there was no fascicular arrangement and the tissue was much more cellular than a normal tendon. This apparently implies that from a morphological standpoint the grafting procedure in rabbits is superfluous as the connective tissue strand which replaces the removed calcaneal tendon resembles this in a number of details and also takes up its function. This seems to be so even in dogs, as in some of our "unloaded tendon grafts" a strand formed between the graft and the muscles. This strand had the same histologic picture as the one described by Conway et al.

In our study the fate of the peroneus longus tendon transplanted into its own bed without a tendon sheath was not investigated. Such a study has been made by Seiffert (1967). He has retransplanted peroneus tendons in dogs after excision of the whole tendon except for a small band at the ankle joint. His morphological observations correspond in detail with those of our study when we transplanted the peroneus longus tendon for the Achilles tendon without its tendon sheath.

From the above observations, it may be said that a tendon transplanted without tendon sheath is involved in a tissue reaction which in all respects resembles that of repair. The transplanted tendon becomes involved in this tissue and subsequently it is completely replaced by the healing tissue which appears whether or not tendon graft is present.

The conditions are very dissimilar in reimplantations into the intact tendon sheath. This procedure does not stimulate more repair than is necessary for the severed regions to heal. In this context it is of interest to point out some of the statements made by Peacock (1967) on the restoration of the gliding function. He believes that a secondary remodelling process occurs which causes certain portions of the scar to undergo collagenolysis so that the scar which connects the tendon graft and the skin re-forms so that normal tendon and dermis ensue. He moreover states that it is inaccurate to believe that adhesions must be broken to restore mobility to the tendon. Peacock has dissected a number of fingers with normal gliding tendon grafts in which this resulted from secondary remodelling so that the portion of scar which lay between the tendon and its surrounding bed ultimately resembled loose connective tissue like that normally found around uninjured tendons (paratenon). It thus appears that environmental factors of yet unknown properties may be of importance in the fate of tendon grafts.

Our results also favour the theory expressed by Peacock (1967). According to him, it may be said that "the best possible tissue to surround a tendon graft is a normal digital theca." This contains few cells capable of producing collagen. Besides, it may be so that the theca contains cells capable of producing an ultrafiltrate from plasma which improves the gliding function.

Against the suspicion that the increased muscular load on the graft when it replaced the Achilles tendon could be responsible for the changed morphological picture is the fact that we saw exactly the same picture whether the tendon was loaded or not.

Regarding the size of the newly formed "tendon" when the peroneus longus replaced the Achilles tendon, the thickness was found to be roughly equal to the original Achilles tendon after 30 days. This, however, was a general observation and not systematically analysed. The increase in size seemed to occur whether the tendon was sutured to the muscle or not. This corresponds with the experiments performed by Conway et al. (1967). It also corresponds with the experiments of Elliot (1965) where he found the thickness of collagen to represent the tension which the tendon had transmitted and it was not necessarily dependent on the strength of the muscle. Passive tension in the tendon as well as increased muscular load results in increased thickness. Elliot performed his investigations on tendons from denervated muscles in young rabbits.

In the literature very divergent opinions have been expressed regarding the fate of a transplanted tendon. Mason & Shearon (1932) and Peer (1955, 1959) are of the opinion that the autologous tendon transplant will survive. An entirely opposite standpoint is taken by, e.g., Skog & Persson (1954) and Flynn et al. (1960). These latter authors believe that the tendon succumbs and only serves as a guiding matrix for ingrowing fibroblasts from the surrounding connective tissue. A third position in this question is taken by, e.g., Imayoshi (1925) and Lindsay & Birch (1964). These authors believe that the peripheral parts of the transplant survive while the central portions are replaced by connective tissue after primary necrosis. Lindsay & MacDougal (1961) and Birdsell, Tustanoff & Lindsay (1966) discuss a process called "reconstitution" which involves a combination of survival and repair. Our results when we reimplanted the peroneus longus tendon in the tendon sheath indicate a total surviving tendon graft. The result when we transplanted the peroneus longus for the Achilles tendon corresponds with the opinion that the tendon transplant is entirely replaced by ingrowing connective tissue. This appears to take place along the earlier position of the mesotendon, the existence of which has previously been reported by Mayer (1916).

Thus our results could perhaps explain the differing opinions. They could to a great extent be related to the different circumstances under which the experimental transplantations have been performed and the response of the host tissue. Further experiments are necessary to confirm this definitely.

SUMMARY

Tendon grafting brings into consideration factors like healing of anastomosis, change in morphologic and functional properties, influence of surrounding tissue, and adaptation to a change in muscle load. The

reparative pattern in tendon grafting much resembles the various stages of wound healing.

A study has been carried out to follow what occurs in the tissue surrounding a tendon graft and the ultimate fate of a thin tendon graft in different types of surrounding tissue.

Experiments have been carried out in 51 dogs on 70 legs. The peroneus longus tendon was used as a graft to replace the Achilles tendon. In other experiments the peroneus tendon was excised and reinserted in its own tendon sheath. Slight loading was exerted on all grafted tendons except in 6 instances.

Histological studies of the grafted areas were carried out after 2, 7, 14, 21, 30, 60, 90, 120, and 180 days.

The results disclosed that loading or non-loading the tendons did not seem to alter the structural development of tissue events.

Histologically it became evident that an inflammatory reaction characteristic of a genuine repair process was instituted and at the time of termination of the experiments with transplanting the peroneus longus tendon into the site of the Achilles tendon a complete replacement of the tendon transplant with ingrowing connective tissue had taken place.

In those experiments where the peroneus tendon was excised and reinserted into its own sheath, there was complete healing at the sites of incision with the tendon completely intact.

These results suggest that tendon transplants act only as a guiding matrix for the repair tissue.

REFERENCES

Adams, W. (1960) *On the reparative process in human tendons. Also a series of experiments on rabbits.* J & A Churchill, Ltd., London.

Birdsell, D. C., Tustanoff, E. R. & Lindsay, W. K. (1966) Collagen production in regenerating tendon. *Plast. reconstr. Surg.* **37**, 504.

Conway, A. M., Dorner, R. W. & Szuckner, J. (1967) Regeneration of resected calcaneal tendon of the rabbit. *Anat. Res.* **158**, 43.

Elliot, E. H. (1965) The growth of tendon after denervation or excision of its muscle. *Proc. roy. Soc., Ser. B.* **162**, 203.

Flynn, J. E., Wilson, J. T., Child, C. G. & Graham, J. H. (1960) Heterogenous and autogenous tendon transplants; an experimental study on preserved bovine tendon transplants in dogs and autogenous tendon transplants in dogs. *J. Bone Jt Surg.* **42-A**, 911.

Flynn, J. E. & Graham, J. H. (1965) Healing of tendon wounds. *Amer. J. Surg.* **109**, 315.

Imayoshi, M. (1925) Experimentelle Untersuchungen über Sehnenregeneration unter anwendung der vitalen Carminspeicherungsmethode nach Kiyno. *Langenbecks Arch. Klin. Chir.* **137**, 143.

Lindsay, W. K. & MacDougal, E. P. (1961) Digital flexor tendons; an experimental study III. The fate of autogenous digital flexor grafts. *Brit. J. plast. Surg.* **13**, 293.

Lindsay, W. K. & Birch, J. R. (1964) The fibroblasts in flexor tendon healing. *Plast. reconstr. Surg.* **34**, 223.

Mayer, L. (1916) The physiological method of tendon transplantation. *Surg. Gynec. Obstet.* **22**, 182, 298, 472.

Peacock, E. E. (1965) Biological principles in the healing of long tendons. *Surg. Clin. N. Amer.* **45**, 461.

Peacock, E. E. (1967) Biology of tendon repair. *New Engl. J. Med.* **276**, 680.

Peer, L. A. (1955) *Transplantation of tissues*. Pt 1. Williams & Wilkins Co., Baltimore.

Peer, L. A. (1959) *Transplantation of tissues*. Pt 2. Williams & Wilkins Co., Baltimore.

Seiffert, K. E. (1967) Biologische Grundlagen der homologen Transplantation conservierte Bindegewebe. *Mschr. Unfallheilk.* **93**.

Skog, T. & Persson, B. H. (1954) An experimental study of the early healing of tendons. *Plast reconstr. Surg.* **13**, 384.