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Substitution of the Anterior Cruciate Ligament: A Long-Term Histologic and Biomechanical Study with Autogenous Pedicled Grafts of the Iliotibial Band in Dogs*

Theo J. G. van Rens, Alter F. van den Berg, Rik Huiskes, and Wim Kuypers

Abstract: This paper reports the experience with anterior cruciate ligament (ACL) substitution by standardized pedicled strips of the iliotibial band, fixed to the tibia and femur with a bone-peg fixation technique. Thirty-two young adult (± one year old) Labrador dogs were used for the experiments (58 transplant knees and six controls). The posttransplantation period ranged from day zero to three years postoperatively. Of the transplants, there were five failures. Forty-seven knees served for gross, photographic, and histologic examination. In 17 knees, the transplants were submitted to tensile testing in an Instron testing machine. The behavior of the ACL transplants was as follows: the transplants became necrotic in a short period of time, but after day four, a process of creeping substitution took place. At 12–16 weeks, a newly formed ligament was seen with a striking macroscopic resemblance to the normal ACL. However, its collagen fibers were coarser and more undulant, and they were fixed to the bony interfaces with Sharpey-like fibers. When these rather good-looking ACLs were subjected to mechanical testing and compared with normal ACLs, the results were less satisfactory. Mechanically, the substituted ACLs were 45% less stiff than normal ligaments, the yield point was at one-third, and the ultimate load was 40% of that of a normal ACL (deformation rate, 5 mm/min). Normal ACLs rupture at their tibial insertion. The transplants ruptured intraligamentarily. A striking finding was that the substituted ligament did not derotate when all the other ligamentous and capsular structures were cut. There was no torsional arrangement of their component bundles. Although the substituted ACL has an excellent histological and macroscopic appearance, its mechanical properties are inferior. **Key Words:** Anterior cruciate ligament—Transplants—Dogs.

[Editor's comments: This beautifully prepared research paper by Dr. Theo J. G. van Rens was presented in part at the Annual Meeting of the Arthroscopy Association of North America in Boston, Massachusetts in 1985. It sheds some light on the rather disappointing results of Dr. John F. Meyers' paper (see pp 155–161) on second-look arthroscopy of previously reconstructed anterior cruciate ligaments. If 40% strength is the best we can anticipate from cruciate ligament reconstruction in humans, we will have to rethink our rationale for performing this operation. Although we know we cannot extrapolate animal experiments and apply them to humans, there is no useful purpose served in performing some animal experimental surgery if we cannot draw some conclusions that also apply to humans.]

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    * *Arthroscopy* is pleased to publish this fine article by Dr. van Rens, but is greatly saddened by the news that he passed away on August 9, 1986.

Virtually all types of collagenous tissue have been transferred in and around the knee joint for intra- and extraarticular ligament reconstruction. The success of these ligament transplants, in partic-
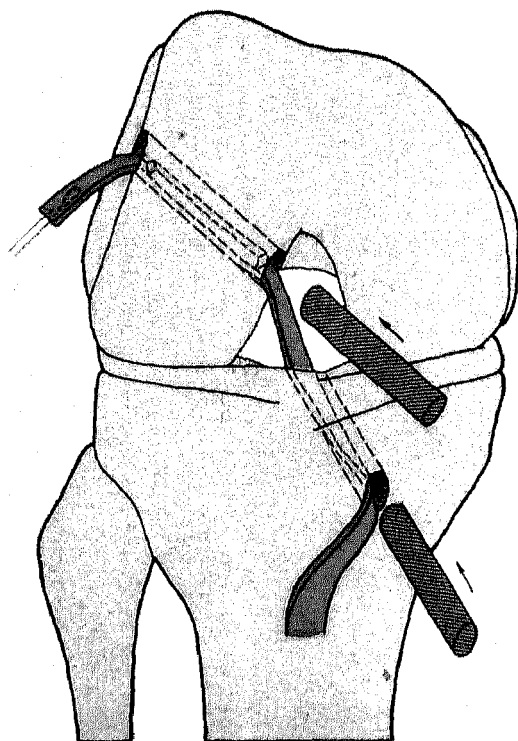
FIG. 1. Schematic drawing of the insertion and fixation of the strip of iliotibial band into the femoral and tibial condyles (bone peg fixation).

ular for the anterior cruciate ligament (ACL), varies so much that still no uniform method of treatment can be recommended. Nearly all transplants, no matter where they derive from or what technique is used, tend to become loose in time.

The results of ACL replacements by means of biological (tendinous or other) material have been so disappointing that to augment biological ligament transplants artificial ligaments of artificial materials are now in the process of being tested, not only in animals but also in humans.

What factors determine this capricious outcome of biological intraarticular transplants? Most reports on ACL transplants describe the technique used and mention the overall clinical results achieved. However, a systematical long-term study with data on the histological characteristics of such a transplant, especially of its anchorage to bone and its mechanical properties, in particular the strength and stiffness of such a graft, are incomplete or difficult to interpret. Most data are fragmentary (1,2),

or were observed in a group too small to provide overall conclusions (3).

Criteria for success or failure vary widely and include subjective parameters such as gait, degree of lameness (in dogs for example), anterior drawer, and macroscopic integrity of the substituted ACL.

To provide more information about substitution of the ACL by a biological transplant and its ultimate mechanical properties, a study was performed in dogs where the ACL was resected completely and replaced by a pedicled strip of the iliotibial band, as described by O'Donoghue in 1963 (4), and 1971 (5), and fixed to the condyles by a peg fixation technique, as described by us previously (6).

We posed the following questions: What happens to the transplant with time? What happens in the bone-transplant interface? Do the mechanical properties of the transplant change with time, and what is the ultimate stiffness, strength, and deformation after 1 year?

MATERIALS AND METHODS

Thirty-two young adult Labrador dogs bred in our Animal Laboratory (64 knees) were available for the experiments.

In 58 knees, the ACL was excised completely and then reconstructed using a pedicled strip of the iliotibial band. A lateral parapatellar incision extending from the medial crest of the tibial tuberosity to the middle and proximal thirds of the thigh was made. The knee joint was opened and the ACL removed at its tibial and femoral insertions. Then, a strip of the iliotibial band (15-cm long, 1-cm wide at its distal part, and 2-cm wide proximally) was dissected free, beginning at the upper end of the thigh and continuing distally up to the band's tibial attachment. This tibial attachment was not divided. The strip of iliotibial band was then passed through an 8-mm bore-hole made with a trephine, from the anterolateral surface of the tibia to the site of the tibial attachment of the ACL in the knee. After passing the strip of the iliotibial band through this bore-hole, the plug of bone remaining in the trephine was again driven into its canal in the proximal tibia fixing the transplant (Fig. 1).

The iliotibial band was then twisted clockwise six times around its longitudinal axis and subsequently

FIG. 2 (facing page). A: Anterior view of the knee. The transplant is twisted around its longitudinal axis and pulled through the borehole in the femoral condyle. B: The situation after completion of the ACL replacement. C and D: X-rays of the knee after wound closure. The reinserted bone plugs are clearly visible (AP and lateral view).

A,B

C,D

passed through a similar canal made in the posterior aspect of the intercondylar notch—the site of insertion of the ACL or the femur lying in the dorsal part of the canal—and fixed in the same way as described for the tibia. Thus, both intra-condylar parts of the transplant were fixed with bone pegs, and a good bone transplant contact was achieved. The remaining portion of the iliotibial band was sutured to the periosteum of the lateral part of the femur and the lateral intermuscular septum (Fig. 2A–D).

The defect in the iliotibial band was closed, leaving a distal part open in order not to create a lateral shift or increased compression on the lateral facet of the patella. After wound closure, the dogs were not immobilized. They limped, or at least did not put weight on the operated leg during the first three weeks.

After this period, rapid weight bearing was noticed, and at four to six weeks, the dogs were running freely in their large kennels at the farm where they were kept.

In these dogs, we investigated the macroscopic and microscopic appearance and the mechanical properties of the transplants.
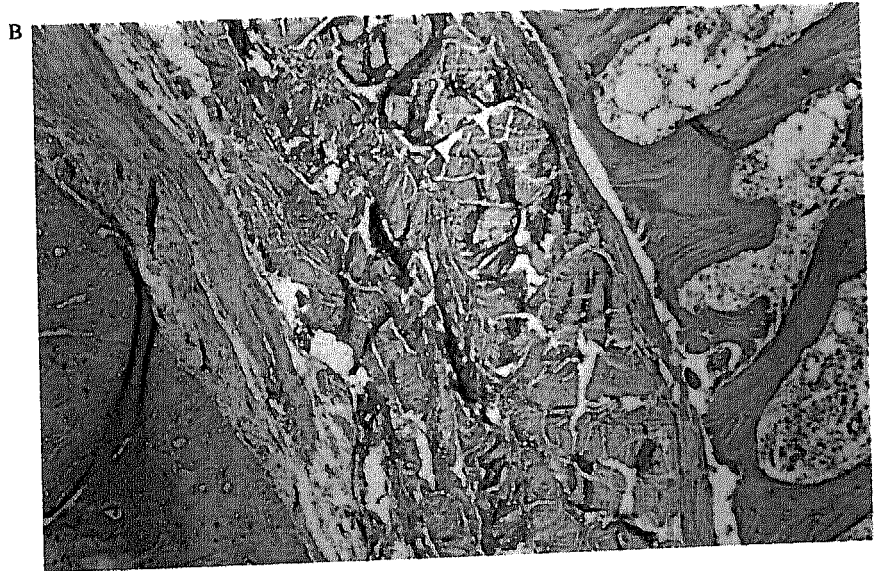
FIG. 4. Day 4: A: Macroscopic appearance: A fibrinous blood clot has formed around the transplant. B: Microscopic appearance: In the right upper corner, the compressed bone plug is visible. In the midportion of the section, the transplant is cut tangentially. The transplant is necrotic (no cells visible). At the left side, the cut trabeculae of the femoral condyle are visible. The bone marrow has a slightly pronounced cellular reaction.

FIG. 3 (*facing page*). A: Hematoxylin eosin (HE) staining of the mid portion of a normal ACL. B: The same section illuminated with polarised light clearly illustrates the collagenous structure. C: HE staining of the femoral insertion of a normal ACL. Ligamentous tissue distally, bone and cartilage cells in the upper half of the section. The blue "tide-line" marks the border between bone and ligamentous tissue (→←). D: The same section, demonstrating that the collagen fibres (Sharpey fibres) from the ligament pass into the bone continuously.

FIG. 5. Day 7: Necrosis of the transplant (left) and massive cellular reaction in the bone marrow adjacent to the necrotc transplant. Mononuclears and giant cells invade the necrotic "ligament."

Group I

Forty-seven knees were used for histologic and macroscopic investigation of the bone transplant junction in femur and tibia, and for investigation of the free midportion of the transplant in the knee.

The animals were killed at Day 0, (2); Day 4, (2); Day 7, (2); two weeks, (2); four weeks, (3)-(1) control; six weeks, (2)-(1) failure; 10 weeks, (4); 12 weeks, (4); 16 weeks, (4)-(1) failure (1) control; six months, (5)-(1) failure; one year, (7)-(1) control (1) mechanical testing; 18 months, (2); and three years, (3)-(1) failure.

Group II

Seventeen knees were used for mechanical testing 1 year postoperatively. Three knees served as controls. There was one failure.

Six knees served as a control group for histology or mechanical testing (three in each group).

At the planned date, the dogs in Group I were killed with an overdose of pentothal. The knees were inspected for the appearance of the transplant, x-rayed, and photographed. They were then prepared for microscopic evaluation, especially the transplant's midportion, and for the place of the insertion on the condyle. The microscopic sections were stained with hematoxylin-eosin (HE), and also with P.T.A. and Van Gieson elastin staining. The HE sections were also studied with polarized light.

The dogs in Group II were subjected to mechanical testing in an Instron testing machine. These dogs were killed at one year postoperation. The knees were tested in tension at a constant rate of displacement to failure after sectioning of all the ligamentous structures and the capsule of the knee with exception of the substitute ACL or the original ACL. These experiments were performed within 2 h after the dogs were killed.

RESULTS

Macroscopy and histology

In Group I, all except four knees have an intact ACL transplant, as illustrated in Figs. 11B and 14B. The knees with a follow-up of >16 weeks in which there was a failure of the transplant show a mild-to-moderate osteoarthritis.

The knees used for the microscopic study were not subjected to mechanical testing, as this would damage the transplant in the early phases of repair and ingrowth, thus influencing our histological data. We decided to perform the mechanical tests at one year, long enough for complete histologic healing of the transplant.

The normal ACL

Figure 3 shows a section through the midportion (A and B) and the insertion place of the ligament into the bone (C and D). The Sharpey fibers continue from the ligament into the bone and the "tideline" marking the borderline between ligamentous tissue and bone is visible in C (see area between arrows).

The ACL transplant

Day 0

Figure 2B illustrates the immediate postoperative appearance.

Microscopically, the bone plug consisting of compressed spongy bone is tightly filling the canal

in the condyle, thus fixing the iliotibial band transplant.

Day 4

Using macroscopy, fibrinous transformation of the blood clot around the transplant is seen (Fig. 4A).

Using microscopy, necrosis of the transplant and slight, mainly mononuclear cellular reaction in the adjacent bone marrow around the transplant and the bone plug is seen (Fig. 4B).

Day 7

The same macroscopic appearance is observed. Microscopically, complete necrosis of the transplant and massive mononuclear cell infiltration from the bone marrow spaces into the periphery of the transplant is seen (Fig. 5). There are many giant cells and osteoblastic activity in the condylar areas.

Day 14

There is more pronounced fibrinous tissue around the intraarticular part of the transplant. Using microscopy, cell ingrowth in the superficial fibrinous layer surrounding the intraarticular part of the transplant, metaplasia of the invading cells in the transplant to fibroblast-like cells, and fibroblastic proliferation can be seen. There was osteoblastic activity in the bone-ligament surface around the bone plugs and the embedded iliotibial band,
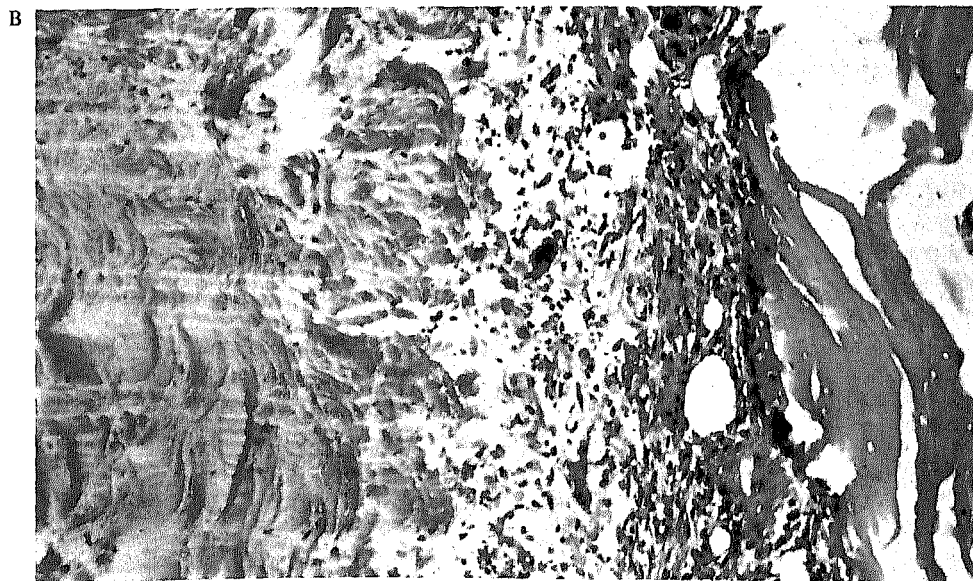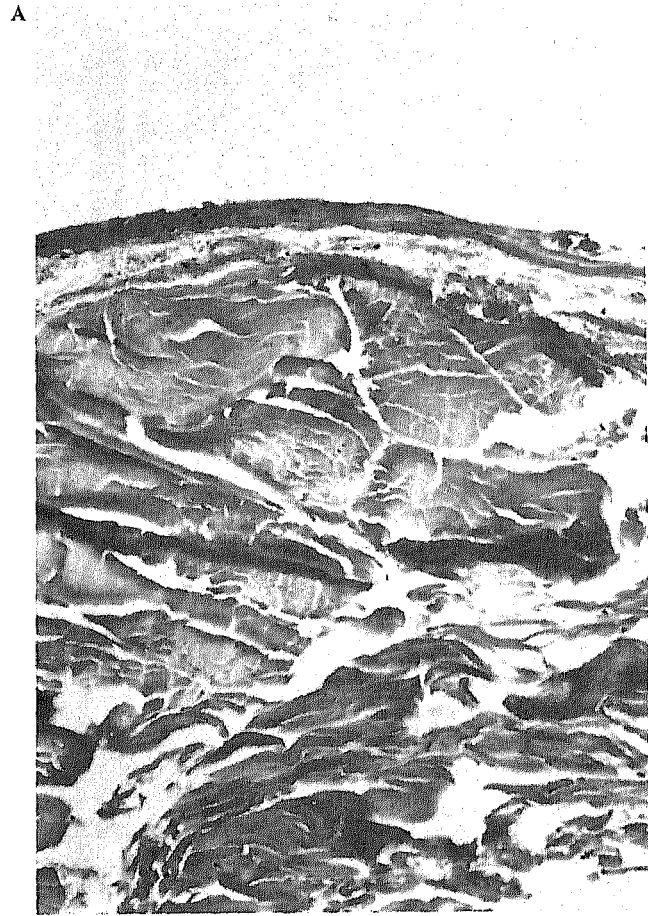

FIG. 6. Day 14: A: Midportion of the transplant. Necrosis. A thin layer of fibrin covers the transplant. In this layer, a cellular invasion is visible. B: Interface bone—ligament. Invasion of mononuclears and giant cells into the necrotic transplant.

FIG. 7. Four weeks: Section through the bone—ligament inter-
face. Compressed bone plug (upper part) and osteoblastic and
clastic activity. Cartilage cells are visible as well. Massive cel-
lular invasion of the transplant and fibres running from the bone
plug into the transplant! (Sharpey fibres?)

FIG. 8. Six weeks: More pronounced fibre formation at the bone
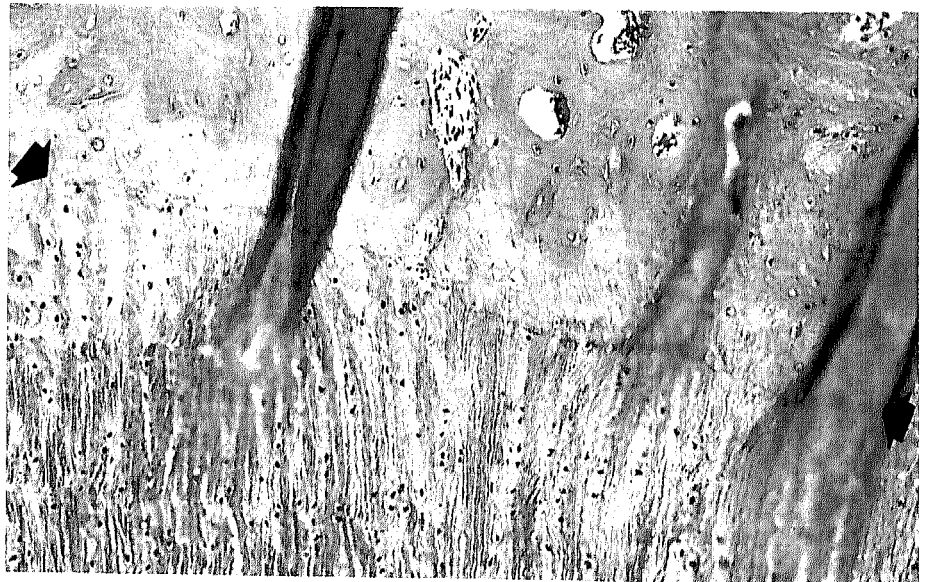—ligament interface. Between the fibres still a cellular invasion.

FIG. 9. 10 weeks: Bone—trans-
plant interface. Clearly visible:
the "tide-line" marking the
border between the body and the
ligamentous area (→←). Active
osteocytes and cartilage cells
near this borderline. Continuous
arrangement of fibrous bands
from this area into the free part of
the newly formed ACL. Still
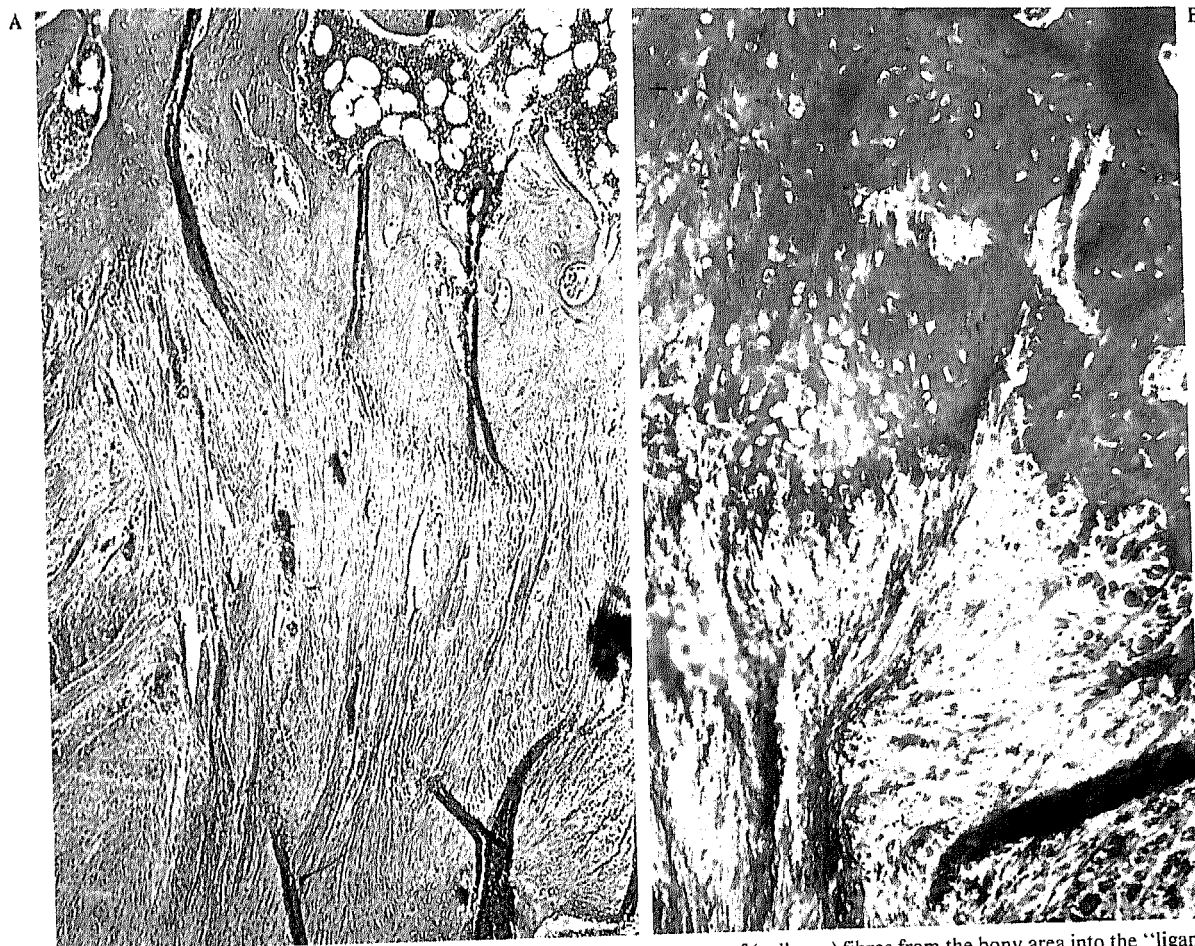many monocytes in between the
fibres.

FIG. 10. 12 weeks: A: Bone—transplant interface. Continuous arrangement of (collagen) fibres from the bony area into the "ligament." B: P.T.A. staining. Dark red: the older bone trabeculae. Light red: the newly formed bone. In between: cartilage cells. Also visible are the fibrous bands running from the bone into the newly formed ligament.

and invasion of mononuclear and giant cells into the transplant (Fig. 6A and B).

Four weeks

Macroscopically, an ACL-like structure with a gelatinous white shine aspect and adhesions with the Hoffa fat pad is seen. Using microscopy, the following are seen: Fibroblastic transformation of the transplant; still cellular activity in the bone marrow spaces and in the transplant; and groups of cartilage cells in the condylar areas, due to metaplasia or enchondral ossification, newly formed bone, and strings of fibrous tissue and collagen fibers running from the spongiosa into the transplant. The fibers resemble Sharpey fibers (Fig. 7).

Six weeks

Macroscopically, the same aspect is seen. Microscopically, further fiber formation with many cells,

mainly mononuclear, interspersed between the fibers is visible (Fig. 8).

10 weeks

Macroscopically, the same aspect is still seen. Microscopically, the transformation of the transplant by creeping substitution into fibrous tissue is nearly complete. The "tideline" marking the borderline between bone and ligamentous tissue seen in the normal ACL is also being formed (Fig. 9).
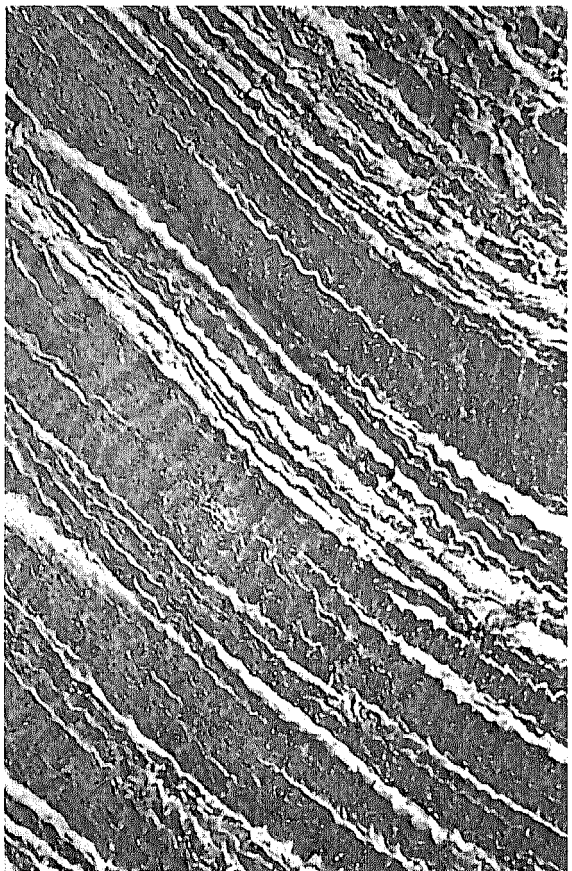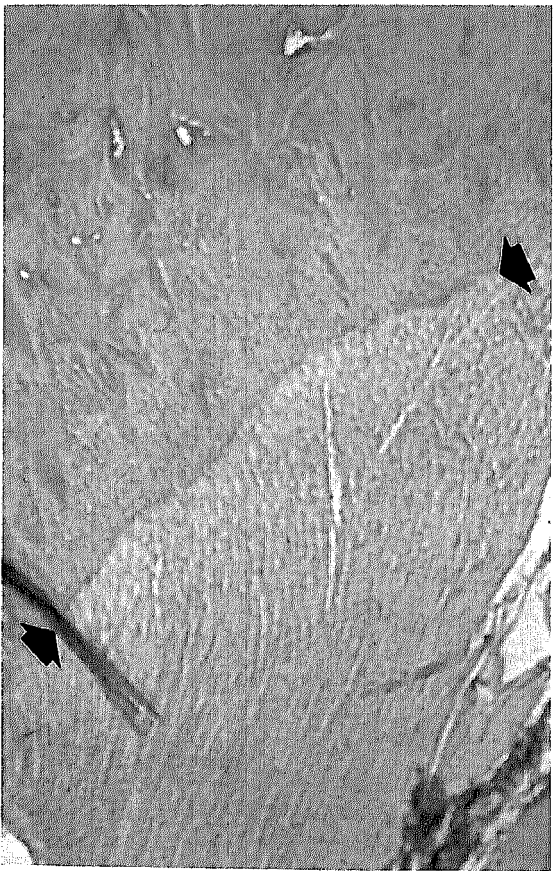
12 weeks

Using macroscopy, a close resemblance to normal ACL is seen. Using microscopy, tendon-like structure containing living cells is visible. In the condylar insertion, parts are surrounded by young newly formed areas of bone. Distinct Sharpey fibers continue from the bone into the transplant (Fig. 10).
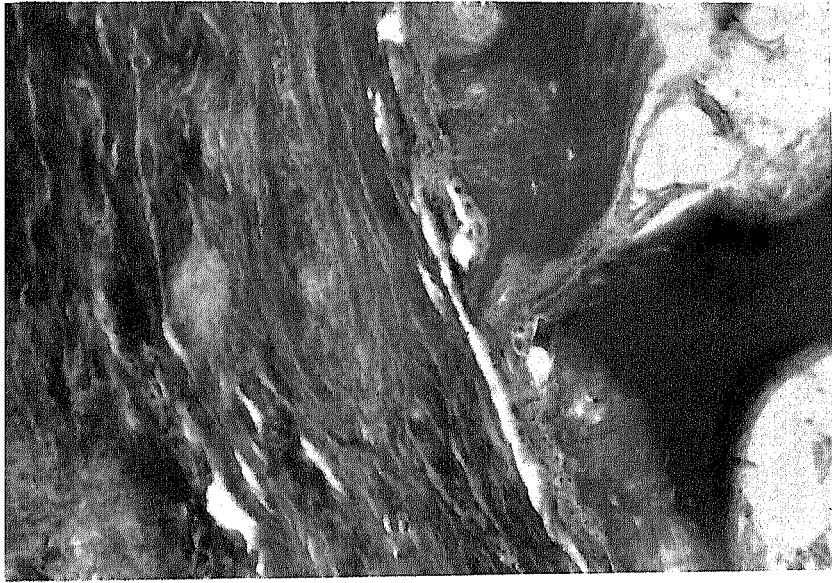
A,B
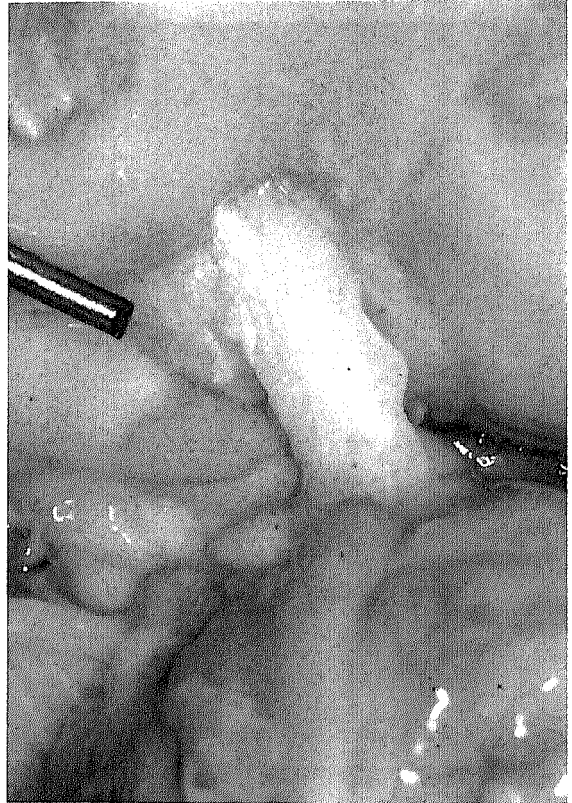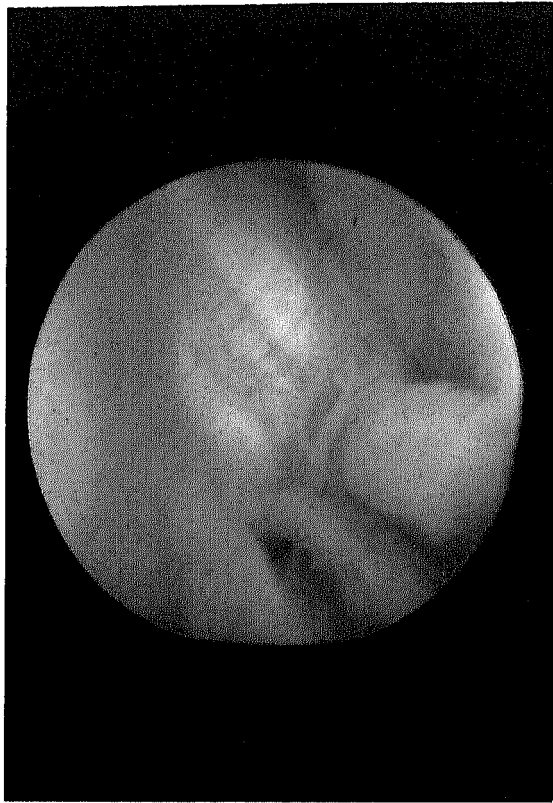
C,D

FIG. 11. 16 weeks: A: Arthroscopic appearance of the new ACL. B: Macroscopic appearance of the same ligament. There is no osteoarthritis. Partly visible: the lateral meniscus. C: Bone—transplant interface. (→←) again marks the "tide-line." D: Midportion of the new ACL. The collagen fibres are coarser and more undulated. E: Van Gieson staining: larger magnification indicating the (red) living cells in the ligament (green).

eks

ere is a normal ACL-like structure (Fig. 11A
B). Microscopically, more cellular ligamentous
with coarse undulant fibers continuing from
ent into bone is seen (Fig. 11C–E).

months, one year, 18 months, and three years
ere are no major differences in the findings
16 weeks. There is a normal appearance of the
substitute, both macroscopically and micro-
ically (see Fig. 14B).

In summary, the following observations were
made: The transplant becomes necrotic in a few
days. However, at the end of the fourth day, a reactive and simultaneous process of cellular infiltration, followed in the next weeks by new bone formation in the contact area of the transplant and ingrowth of young connective tissue, is seen. Synovial lining of the free part of the transplant takes place by cellular infiltration along the fibrinous mass surrounding the intraarticular part of the transplant.

12. Six months: Failure of
C. transplant. Pronounced
arthritis. Partial obliteration
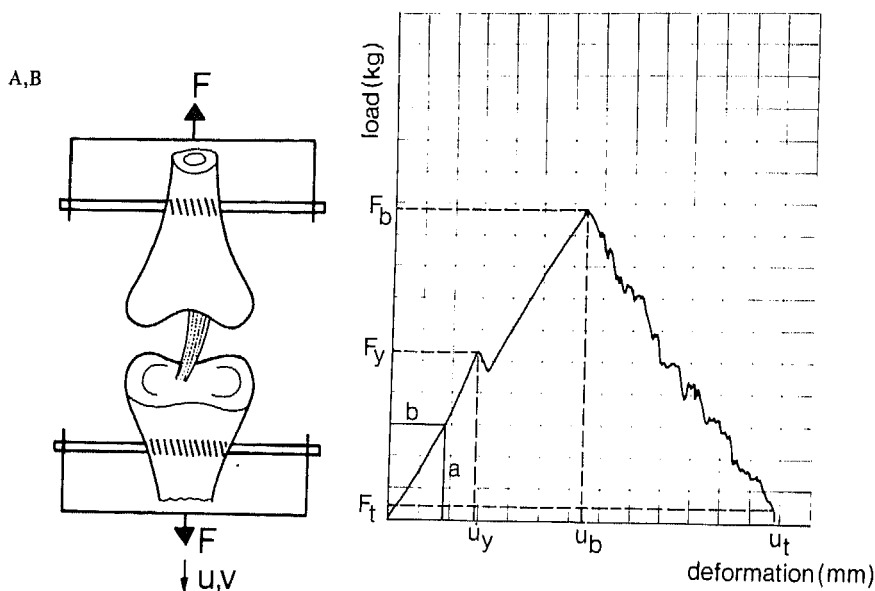e intercondylar femoral
l Synovial hypertrophy. The
is visible.
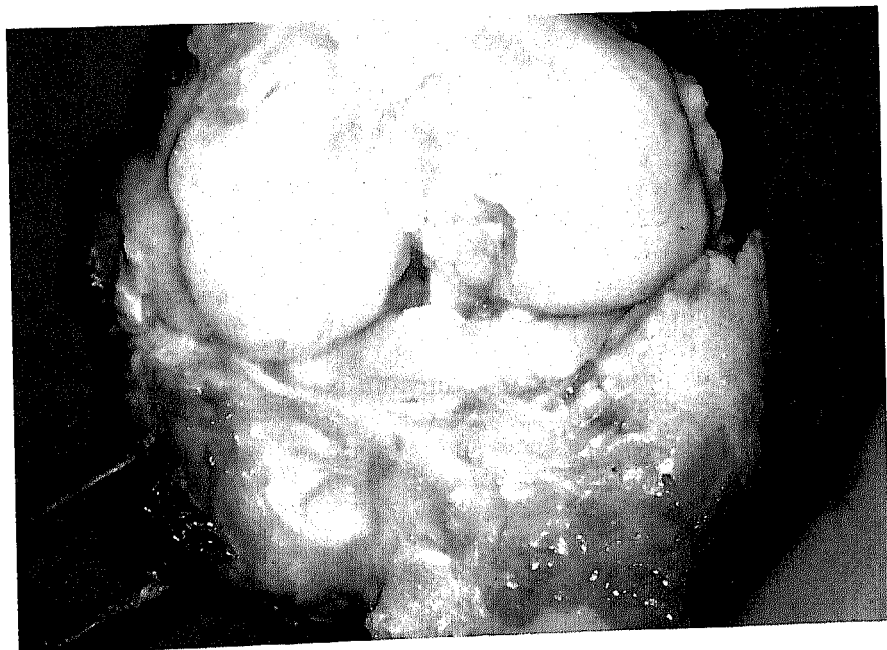
FIG. 13. A: Schematic drawing the set-up in the Instron testi machine. Longitudinal tractic was applied. F = distractic force (kg); U = displaceme elongation (mm); V = stainra (mm min.). B: Schematic drawii of the curves acquired indicatii the qualifications such as yie load and deformation, ultima load and deformation. The stil ness of the ligament is indicate by the steepness of the initial pa of the curve. F_y = yield load; t = yield deformation; F_b = ult mate load; U_b = ultimate defo mation; F_t = end load; U_t = du tility.

Gradually, the transplant is substituted by newly formed dense fibrous tissue with collagen fibers that resemble Sharpey fibers at the bone transplant junction. This process is completed in ~16 weeks. From that point on, the transplant closely resembles a normal ACL.

However, compared to the normal ACL, the collagen fibers have a more coarse and undulant appearance, are more or less in a parallel arrangement, and there is a more cellular and hypertrophic synovial lining intraarticularly.

Examination up to three years postoperatively showed no signs of osteoarthritis when the transplants were intact. Osteoarthritis occurred in four knees where the substitute failed (one at six months, two at one year, and one at three years) (Fig. 12).

Mechanical tests

Seventeen knees (Group II) were used for mechanical testing. There was one failure.

The dogs are killed one year postoperatively. The tibia and femoral condyles are transfixed with threaded Steinmann pins. Subsequently, all the ligamentous and capsular structures are cut, except the ACLs in the two control knees and the substitute ACLs in the transplanted knees.

The specimens are then placed in an Instron testing machine and subjected to tensile testing. The deformation rate was 5 mm/min (Fig. 13A and B).

After transsection of the knee ligaments, excep the ACL, an interesting phenomenon is seen whe the femur is fixed and the tibia allowed to han, freely at the ACL. In normal ACLs, a rotation o the tibia of ~180° along the longitudinal axis of th ACL is seen, thus demonstrating that a detortio took place in the ACL. This detortion or unrollin, effect is not seen in the ACL substitute (Fig. 14/ and B).

Another constant feature is that normal ACL: always ruptured at the tibial insertion, wherea: substitute ACLs ruptured intraligamentally (Fig 15A and B).

The force-displacement curves of both types ol ligaments are also different, the transplants being less stiff, which is demonstrated by a less steep distraction curve (Fig. 16).

The yield load and the ultimate load are also much lower than in the normal ACLs. The differences in yield deformation and the ultimate deformation are less pronounced (Figs. 17A and B, 18A and B).

The mean values for the transplants and the normal ACLs show that the ACL substitutes have an average stiffness of 45%, as compared with normal ACLs. The yield point is at one-third of the normal ACL. The ultimate load for rupture of the ACL substitute has mean values of ~40% of the normal ACL. The mean values for the yield deformation and the ultimate deformation are given in Figs. 17A and 18B.
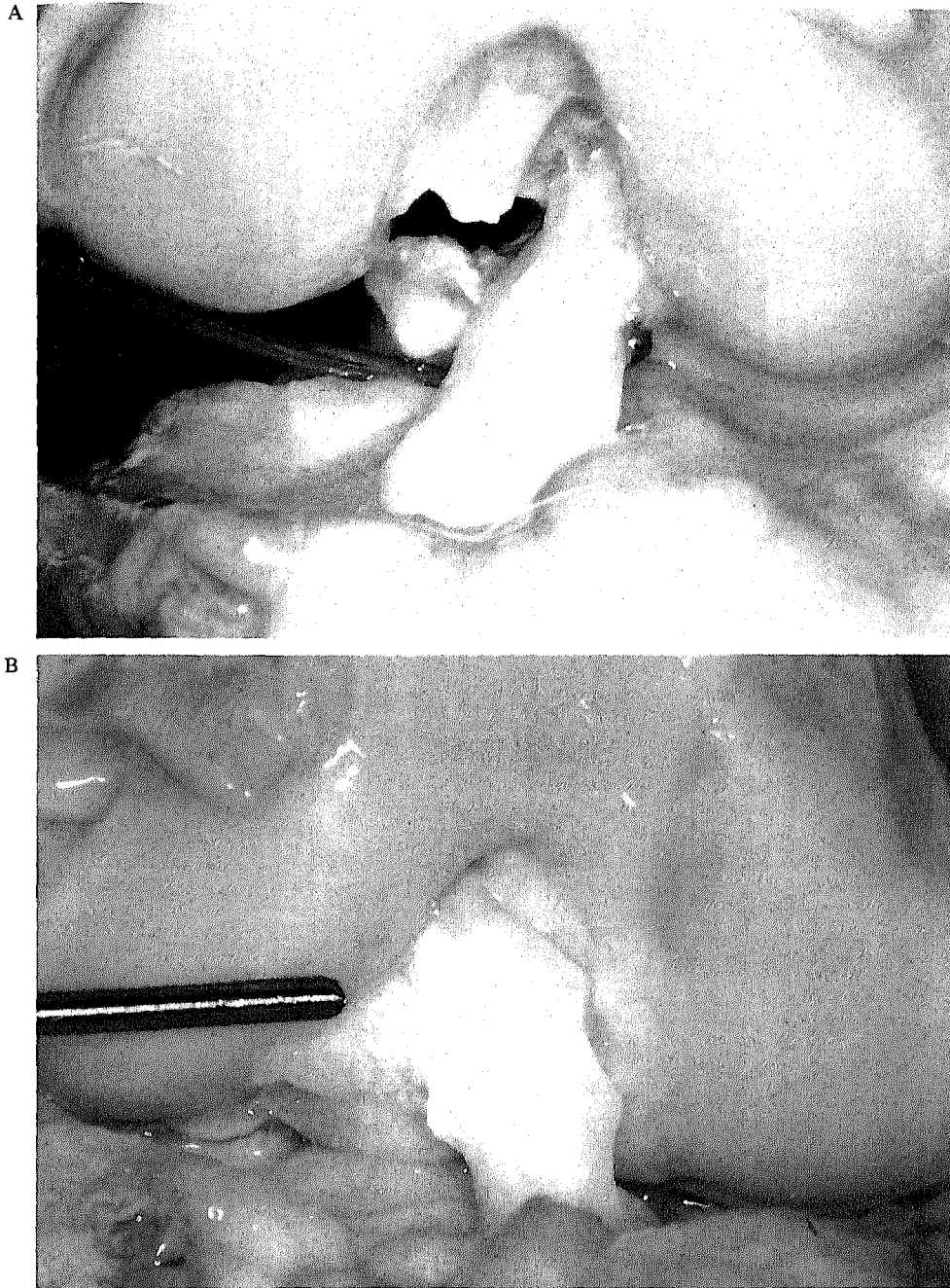
FIG. 14. One year, before mechanical testing: A: Normal ACL after transsection of all other ligaments and the capsule. Notice the torsion in the fibres in the ligament. B: Newly formed ACL. Notice the lack of osteoarthritis, and also the lack of torsion of the fibres in the new ACL. They run in a parallel range from the femoral to the tibial attachment.

DISCUSSION

There is little detailed information in the literature regarding the behavior of ACL transplants, other than the fact that good results are obtained in 44–72% of the cases (7). In our study, we hoped to provide further information on the fate of ACL re-construction by closely following the gross and microscopic appearance of the reconstructed ligament over a three-year period, and by Instron testing of the transplanted ligament and comparing this to a control group.

Our data agree in some respects with the studies by O'Donoghue et al. (5), Loeffler (2), and Alm and
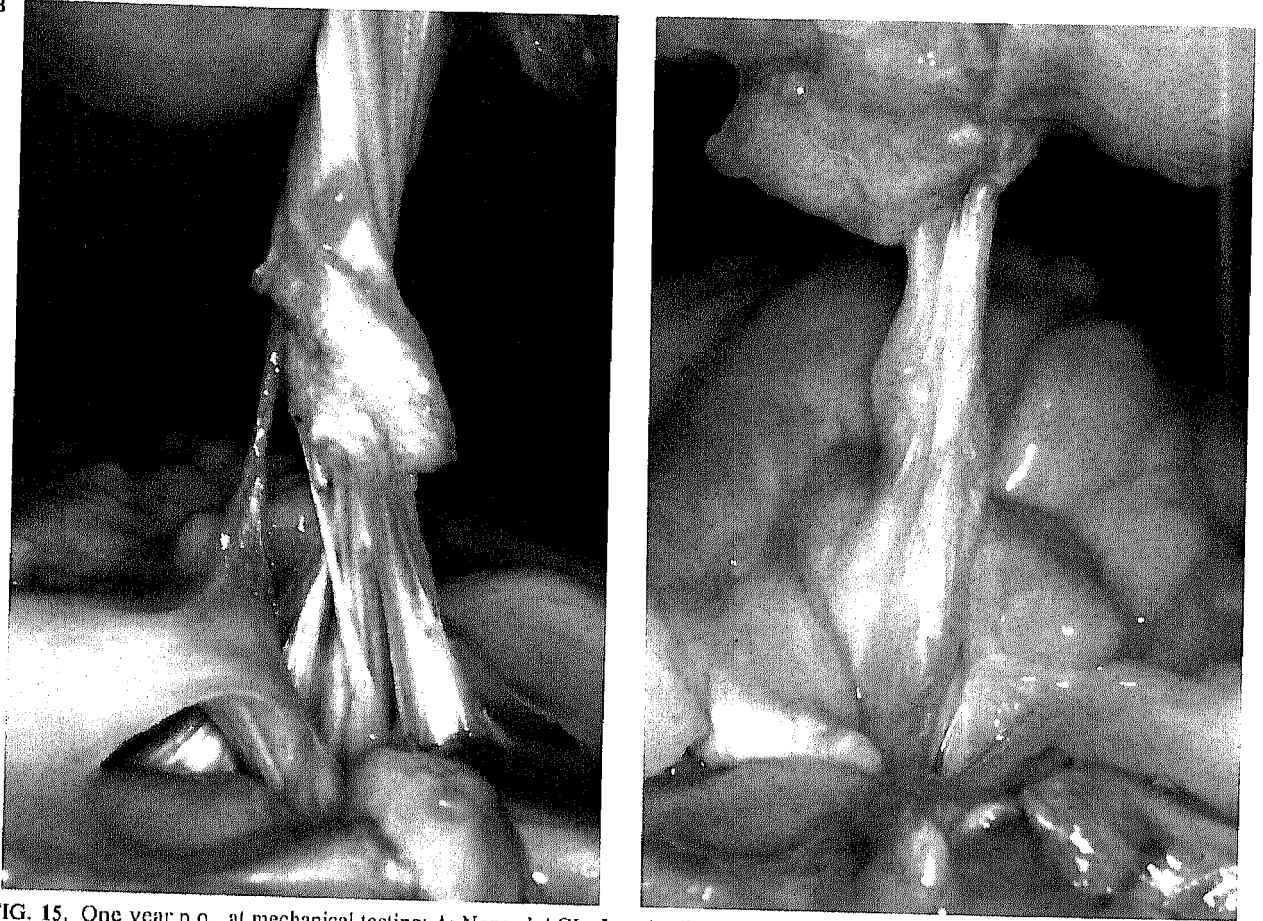
A,B

FIG. 15. One year p.o., at mechanical testing: A: Normal ACL. Longitudinal traction. Disruption from the tibial attachment. B: Transplanted ACL. Interligamentary disruption at the same longitudinal distraction.
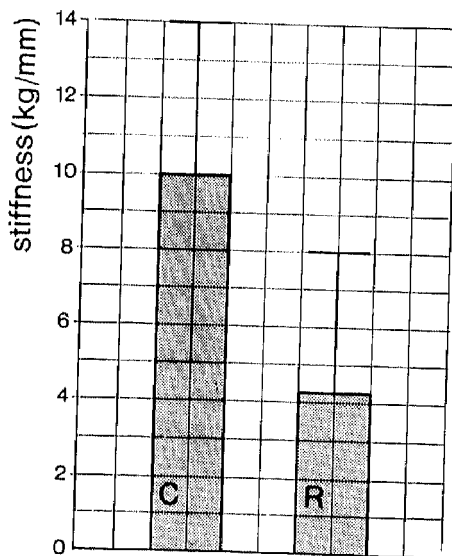

FIG. 16. Stiffness of the ligaments. C, normal ACL; R, transplant.

Stromberg (3). These authors, however, experienced more failures of the transplants than were found in our dogs, probably due to the fact that we had better immediate fixation of the grafted ligament because we placed bone plugs in the condyles. Although our reconstructed ligaments had a reduced stiffness, as compared with the normal ligament, and ruptured at a lower distraction force, the knees were sufficiently stable so that they did not develop osteoarthritis, which occurred when the transplants failed.

The results of reconstruction of the cruciate ligament in humans is still controversial, and too few cases have been followed for a sufficient period of time. Perhaps some of the less-than-satisfactory results reported in humans are due to inadequate fixation of the graft, which we were able to improve by bone peg fixation. The fact that we were unable to achieve anything close to normal strength with
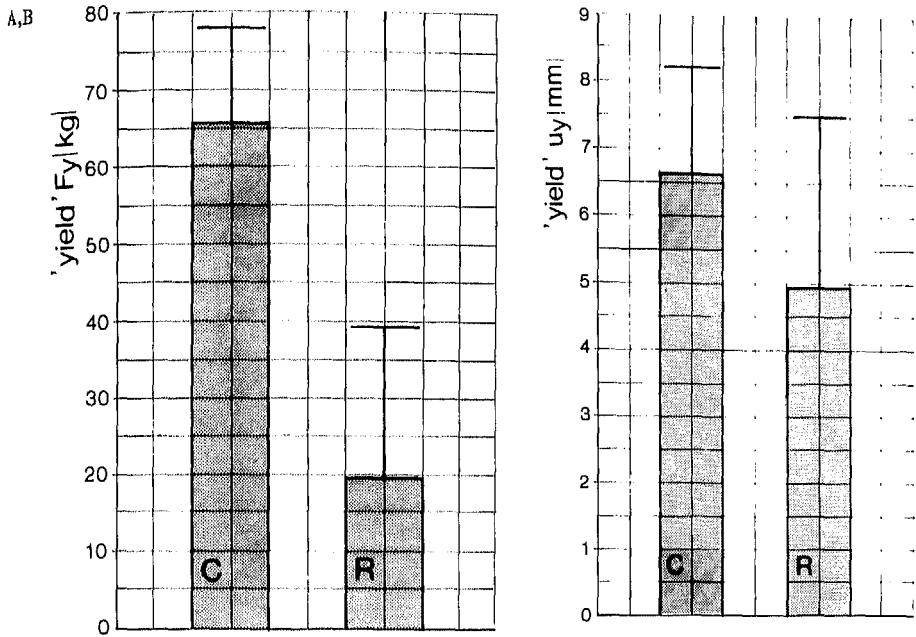
FIG. 17. A: Yield load, the force at which the first bundles in the ACL rupture. C, normal ACL; R, transplant. B: Yield deformation.
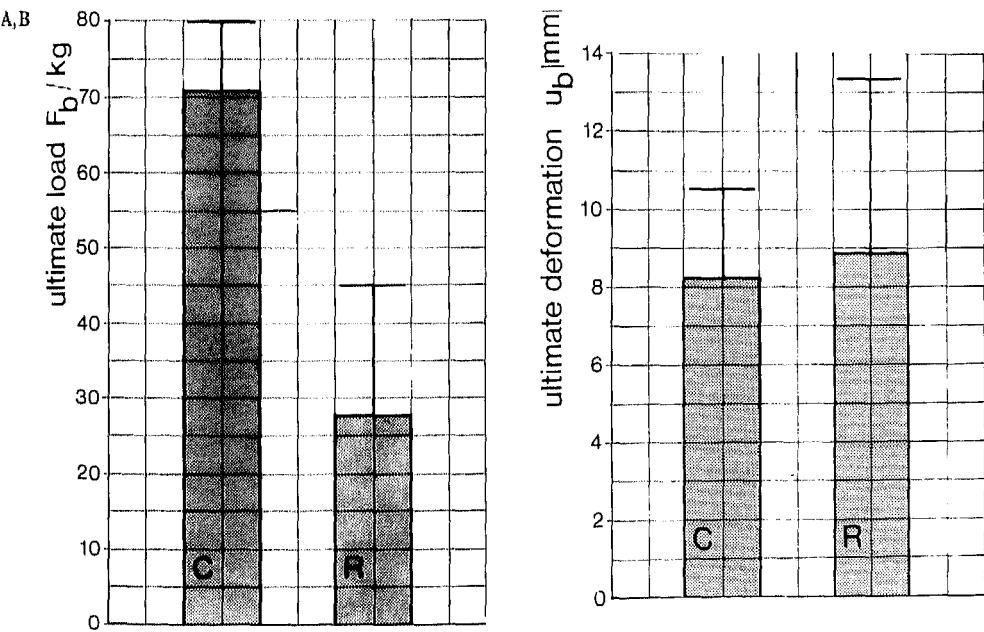

FIG. 18. A: Ultimate load of the ligaments. B: Ultimate deformation. C, normal ACL; R, transplant.

our intraarticular grafts suggests that our results might be improved with an extraarticular graft, possibly using artificial resorbable grafts to support the biological ligament transplants.

Noyes et al. (8) and Butler et al. (9) compared the strengths of various biological substitutions, such as patellar tendon graft, fascia lata, and semitendinosus with each other, and then compared them with the strength of the normal ACL. It is not known whether their values will be the same one year after implantation. The mechanical properties will no doubt be influenced by the operative mechanical factors. Our data show that the initial strength of the graft will change as it is replaced by creeping substitution, and a completely new ligament is formed. It is possible that this also occurs in human transplantation. We attempted to assess the strength of the iliotibial band that we used and

compare it with the normal ACL. However, we were unable to fix the graft firmly enough to test it in the Instron machine. We feel that the strength of the initial graft will differ in time after transplantation. The new ligament formed by creeping substitution will have less favorable mechanical characteristics.

CONCLUSIONS

Substitution of the ACL by a pedicled strip of the iliotibial band, and with good contact in the bone tunnels, will not result in the formation of a normal ACL.

The ACL transplants become necrotic in a very short period of time, but are revitalized by creeping substitution. It takes at least three months before this process is finished, the collagen fibers have matured, and new Sharpey-like fibers are formed in the bone-transplant interface.

The substituted ACLs do have inferior mechanical characteristics when compared with the normal ACL. They do not have the normal torsional arrangement of the component bundles. They all rupture intraligamentarily, whereas in our setup for tensile testing, the normal ACLs ruptured at their tibial insertion. Their stiffness is 45%, the first bundles rupture at one-third, and the ultimate load for rupture has mean values of 40% of that of a normal ACL.

After three months postoperatively, failure of the transplant always resulted in osteoarthritis of the knee with marked narrowing of the femoral notch.

These data were derived from long-term (up to 3 years) animal experiments (Labrador dogs). It is very likely that substitution of the ACL in humans will take place in the same manner.

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