THE EFFECT OF IMMOBILIZATION ON LIGAMENTOUS HEALING AND STRENGTH OF THE MEDIAL COLLATERAL LIGAMENT OF THE RAT KNEE

by

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ABSTRACT

The purpose of this study was to determine the effects of varying periods of immobilization on ligamentous healing and strength in a rat experimental model. Sixty-one mature male Wistar rats were used. The left knee medial collateral ligament was surgically exposed, divided, and repaired. The rats were randomly placed into one of four groups: Group A, no immobilization, Group B, 2 weeks' immobilization, Group C, 6 weeks' immobilization, and Group D, 10 weeks' immobilization of the operated limb. The right knee served as a control. The ligaments were studied histologically and biomechanically at 2 weeks, 6 weeks, 10 weeks and 20 weeks post-operatively. Histologic samples were objectively evaluated with the light microscope using a Maturity Index Score and Scale that were devised based on the numbers and orientation of the fibroblasts and the amount and orientation of the collagen fibres. Ligament-bone preparations were studied using an Instron material testing machine to determine the biomechanical properties of the ligament until failure.

Utilizing the Maturity Index Score and Scale, it was shown that Group A, with no immobilization, matured more rapidly than the other groups, and achieved full maturity at 20 weeks post-operatively. The other groups all showed a retarded rate of healing while immobilized. The electron microscopic study supported this data by demonstrating the level of metabolic activity of the fibroblasts which decreased with increasing maturity and by demonstrating that the size, amount and orientation of the collagen fibers increased with mobilization.

The biomechanical testing showed that at 2 weeks post-operative, Group A had achieved a strength which was 46% of controls while Group B was only 29% of controls ($p = 0.055$). At 6 weeks Group A was 65% of controls, Group B was 56% of controls and Group C was 39% of controls ($p = 0.0004$). At 20 weeks Group A was 83% of controls, Group B was 71% of controls, Group C was 66% of controls and Group D was 48% of controls ($p = 0.0005$). Group A was 71% stronger than Group D at this time, indicating that the healing medial collateral ligament attained a greater strength and histologically matured more rapidly if mobilization is begun immediately.

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INTRODUCTION

A. Current Management Following Knee Ligament Injury or Reconstruction.

Since the time of Hippocrates, physicians have been advocating immobilization of damaged ligaments and joints with various forms of plaster casts, splints and bandages. However, the optimal period of immobilization following a knee ligament repair or reconstruction is controversial [5,9,14,26,30]. Various authors (Palmer, O'Donoghue, Noyes) have advocated periods of immobilization of 6-12 weeks after knee ligament repair or reconstruction [5,24]. This is followed by an extensive period of mobilization and physiotherapy. Other authors (Alm[5], Haggmark[14], Dehne[9] and Perkins[30]) have advocated more controversial methods of post-operative, or post-injury management. Alm[5] feels that four weeks of plaster immobilization is sufficient while Haggmark[14] believes that immediate cast brace applications is the preferred method of management. Both Dehne[9] and Perkins[30] believe that no immobilization is necessary. Yet there is no published well designed experiment studying the effects of varying periods of immobilization on the healing ligament. Authors, such as Clayton and Weir, have stated that "attempts were made to determine the effects of immobilization on the rate of healing, but, since it was difficult to maintain satisfactory immobilization by plaster, the results were discarded"[8].

B. The Effects of Immobilization.

The claims of rest have been advocated, in the past, by such notable surgeons as H.O. Thomas, John Hilton, and Sir Reginald Watson-Jones[30]. They regarded rest as a matter of noninterference as in the case of John Hilton or

as a positive treatment as in the teaching of H.O. Thomas. "He believed and preached that rest had magical properties"[30]. However the harmful effects of prolonged rest and immobilization have been overlooked by these great men. George Perkins has pointed out the damage to the knee joint when immobilizing a lower extremity in the treatment of a tuberculous hip[30]. Numerous investigators have demonstrated the deleterious effects of immobility on bone, joints and soft tissue structures in both animals and man[1-3,9,10,14,19,20, 22,24,26,30,32,33,35,36,38].

Noyes[26] has demonstrated a decrease in bone density of immobilized bones. He has also demonstrated subperiosteal bone resorption at sites where ligaments of the knee insert directly into bone through periosteum leading to a higher incidence of ligament failure at these sites following immobilization. This applies to the tibial insertions of the medial and lateral collateral ligaments of the knee. The Femoral attachments of these ligaments and the attachments of the cruciate ligaments are through a zone of fibrocartilage which did not appear to be significantly affected by the immobilization process. He also found a significant decrease in the strength of the normal anterior cruciate ligament to 61% of controls after being immobilized for 8 weeks. The strength of these ligaments was still only 91% of controls after 12 months of reconditioning.

Evans[10] studied immobilization and remobilization of rat knee joints and found that after prolonged immobilization (after 30 days) contractures of the muscles of the leg and of the capsule of the knee joint lead to a restriction of motion. He found a proliferation of intracapsular connective tissue and the formation of a large number of intra-articular adhesions. He also documented major irreversible cartilage alterations including matrix fibrillation, cleft formation and ulceration with adjacent subchondral lesions. These

cartilage changes were present at as early as 30 days of immobilization and were at areas of tibial-femoral contact and patello-femoral contact.

Using histochemical techniques Akeson[1,2,3] and Woo[36] have documented the response of the connective tissues around the knee to varying periods of immobilization. They demonstrated a significant loss of chondroitin-4 and -6-sulphate, hyaluronic acid, and water in periarticular connective tissues of immobilized knees. They found that the total amount of collagen remained constant but that there was an accelerated rate of turnover of this collagen and that immature collagen was being formed. Their conclusions were that the lubricating properties of hyaluronic acid probably contribute to the ease of motion between adjacent fascial, capsular and ligamentous structures. They postulate that "motion of connective tissue would appear to inhibit the contracture process through several mechanisms: a) stimulation of proteoglycan synthesis, thereby lubricating and maintaining a critical distance between existing fibers; b) ordering (rather than randomizing) the deposition of new collagen fibers so as to resist tensile stresses; c) prevention of anomolous crosslinks in the matrix by preventing a stationary fiber-fiber attitude at intercept points"[36].

Other authors have demonstrated the beneficial effects which motion has on the integrity and strength of ligaments[9,10,14,20,22,24,26,30,35,38]. Laros[20] found that at ligament-bone junctions in dogs existing at various levels of physical activity, strength diminished as physical activity diminished and this decrease was prevented by exercise. Tipton[35] also found that immobilization of a normal rat knee joint lead to a decrease in ligament strength and that forced active mobilization lead to an increase in this ligamentous strength. Zuckerman[38] compared ligament strength of rats subject to various activity levels and found that in the forced exercise group (swimming

or running) there was a significant increase in the ligament strength.

Noyes[26] has also documented the effects of reconditioning of immobilized ligaments showing an increase in strength with reconditioning but with only partial recovery after 8 weeks of immobilization followed by 20 weeks of reconditioning. He also believes that mechanical forces have a favorable influence on healing properties by affecting the alignment and orientation of collagen fibers as well as stimulating the maturation phase of the healing process. He states that the specific details as to the timing, magnitude and mode of application of mechanical forces to achieve such effects are not known.

C. Normal Ligament.

When examined histologically, ligament can be seen to consist mainly of parallel collagen fibers with small numbers of cells interspersed between the collagen fibers. Utilizing the electron microscope these collagen fibers can be seen to have a characteristic regular crossbanding with a periodicity of 640Å. This crossbanding is the result of the overlapping of the three basic units of tropocollagen which are composed of amino acid chains and twisted into a left-handed superhelix with a pitch of 28.6Å[12,15]. The tropocollagen filaments have a high tendency to fibril formation which occurs extracellularly and in a manner where they are arranged parallel to each other.

Situated between the collagen fibers are the cells of the ligament, the immature fibroblasts and the mature fibrocytes, as well as occasional capillaries. The fibroblast is responsible for the production of the tropocollagen filaments as well as for the production of the small amount of ground substance seen within the ligament[15]. This consists of the protein polysaccharides, hyaluronic acid and chondroitin sulphate.

D. Cytology of the Fibroblast.

The fibroblast is an active cell engaged in the production of collagen

and connective tissue matrix. Its dormant phase is the fibrocyte. Histologically it can be seen that the fibroblast contains all of the intracellular organelles associated with a metabolically active cell. The most distinctive feature of the fibroblast is the highly developed rough endoplasmic reticulum (r.e.r.)[31]. This consists of flat or dilated sacs or channels that occupy approximately 35% of the volume of the cell. It is ribosome studded and is responsible for protein synthesis. The r.e.r. is associated with a well developed, randomly dispersed golgi apparatus which is a secretory complex responsible for carbohydrate synthesis and the packaging of proteins and enzymes. The fibroblast also contains numerous large mitochondria for energy production. Other features include a large ovoid nucleus with one or more prominent nucleoli as well as vesicles, lipid droplets and cytoplasmic filaments.

E. Ligament Healing.

Like most other tissues in the body, ligament healing can be divided into three overlapping stages of repair: the inflammatory phase, the proliferative phase and the remodelling phase[15,22,31].

The first phase is the inflammatory phase which lasts four to seven days[15,30]. Initially there is bleeding, exudation and the formation of a fibrin clot which will act as a scaffold for early repair. The ends of the ligament become edematous and there is an invasion of inflammatory cells into the wound. These consist of polymorphonuclear leucocytes followed by monocytes and macrophages. These cells are responsible for cleaning up necrotic material and cellular debris. As early as three or four days, undifferentiated mesenchymal cells and fibroblasts can be seen migrating into this area. This signals the beginning of the proliferative phase which overlaps with the inflammatory phase. The invasion of large numbers of fibroblasts is

accompanied by an ingrowth of capillary buds and the beginning of collagen synthesis. Collagen synthesis accelerates and a ligament callous is formed[22]. This phase of healing is reported to last 20 to 45 days in tendon healing[22].

The third phase of healing is the reorganization or remodelling phase. Here the ligament callous is reorganized into a structure resembling normal ligament in terms of collagen amount and organization as well as cell population. The fibroblasts become less active metabolically and are fewer in number. They gradually align themselves along the longitudinal axis of the ligament or tendon in between the collagen fibers. The collagen fibers also become aligned along the longitudinal ligament axis and are larger in size[12,22,31]. The length of this phase has been reported to be between 60 and 360 days in length in tendons of different species of animals[22].

F. Tensile Properties.

The tensile properties of tendon healing have been documented by Mason and Allen[22]. They found three phases of tensile strength: 1) A phase of rapid diminution of tensile strength corresponding to the phase of exudation and fibrous union and lasting approximately five days. 2) A phase of increasing tensile strength to reach a plateau at about the sixteenth day. This corresponds to the phase of fibroplasia or the proliferative phase. 3) A second phase of increasing tensile strength which corresponds to the phase of maturation and remodelling. This phase begins between the nineteenth and twentyfirst days and continues until maturity.

The structure responsible for the tensile strength of ligaments and tendon is the collagen. It has been reported that a collagen fiber 1mm. in diameter requires a load of from 10-40 kg. to disrupt it[12]. It is felt that the cross-links between the parallel collagen fibers are the key factor in

this strength.

G. The Role of Function in Ligament Healing.

The role that function plays in ligament healing remains controversial. Noyes[26] believes that mechanical forces have a favourable influence on the healing properties of ligament by affecting the alignment and orientation of the collagen fibers. However, he does not support this with scientific data. Moreover, he also believes in immobilizing ligaments post-operatively for at least a six week period. Hirsch[15] states that "partial immobilization after tendon surgery is mandatory or suture insufficiency will ensue". Mason and Allen[22] summarized published experimental data on the role of function in tendon healing and found that tendons healed and regenerated into normal appearing tendons "when subjected to tension"[22].

PURPOSE OF STUDY

The purpose of this study was to determine the effects of immobilization on ligament healing of a rat medial collateral ligament. These effects were to be evaluated histologically and biomechanically. For histologic evaluation, an objective scoring system was devised to be used with the light microscope. The electron microscope was used for further evaluation and for verification of the objective scoring of the ligament healing. For biomechanical evaluation, the ligament was tested until failure and the resulting load-deformation curves analayzed.

The rat was chosen as an experimental model because its knee closely resembles the human knee anatomically. The only significant differences are the presence of ossified menisci in the adult rat and the habitually flexed position of the knee of the rat.

MATERIALS AND METHODS

A. Population and Procedure.

Sixty-one mature, male Wistar rats were used in this study providing 122 knee preparations. The average weight of the animals at the time of operation was 405 grams. The operative procedure for all animals was identical. The rat was anesthetized with an intraperitoneal injection of Sodium Pentobarbitol at a dose of approximately 50 mg/kg body weight. The left hind limb was shaved and then cleaned with an alcohol solution. The operative procedure was performed under clean but not sterile conditions. The rat was placed on an operating board with the knee held in 90^{o} of flexion and exposing the medial surface. A longitudinal incision 1.5 to 2.0 cm in length was made over the medial aspect of the knee joint. Using a dissecting microscope, the medial collateral ligament was exposed and then completely divided at the level of the knee joint. The ligament was then repaired with a horizontal mattress suture of 7-0 "Ticron"* on an atraumatic needle. The ligament edges were opposed but not placed under excess tension. A single mattress suture appeared to adequately oppose the tissues without compromising the ligament integrity. Tissues were kept moist at all times with an isotonic saline solution. The wound was then closed in layers, the skin being closed with 4-0 Dexon*.

Following surgery the rat was randomly placed into one of four groups with varying periods of immobilization: Group A (17 rats) had no immobiliza-

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tion of the operated limb. Group B (16 rats) had two weeks immobilization of the operated limb. Group C (17 rats) had 6 weeks immobilization of the operated limb. Group D (8 rats) had 10 weeks immobilization of the operated limb. In addition there were 3 rats (Group Z) that were studied biomechanically only at the time of surgery (time zero). These served as an indication of the strength of the repair itself. In all cases, the right knee served as a control and had no surgery or immobilization.

All rats were kept in individual cages 18 cm x 18 cm x 25 cm and were fed on a standard diet of rat chow and water ad libitum. The rats were studied at time periods of 0,2,6,10 and 20 weeks post-operative using histological and biomechanical techniques.

B. Immobilization Procedure.

Because of the anatomy of the rat and their constant position of knee flexion, immobilization with a plaster of paris cast alone is difficult. Therefore, following the operative procedure, two .035 inch diameter stainless steel Kirschner wires were inserted in the limb, one in the mid femur and one in the mid tibia. The Kirschner wires penetrated both sides of the leg (Fig. 1). A plaster of paris cast was then applied incorporating the Kirschner wires for fixation to the leg. The knee was immobilized at an angle of approximately 90 degrees of flexion and the foot was left out of the cast to allow the rat to have relatively unhindered activity. Ninty degrees of knee flexion was chosen as this is the physiologic resting angle of the knee in the rat as reported by Tipton[34].

While still wet, the plaster of paris was sprayed with tincture of Benzoin to give the cast a bitter taste, discouraging the rats from eating their casts as they are known to do. This method proved very effective. Casts were changed at two weeks and at six weeks post-operative to ensure

a good fit on the atrophied leg minimizing any motion. Casts were also reinforced or changed more frequently if necessary because of excess wasting of the leg and partial destruction of the cast. The average weight of the cast and Kirschner wires was 25 grams or approximately 6% of body weight. Casts were removed by soaking them off in warm water with the rat under pentobarbital anesthesia. Following cast removal, the rats were either studied or allowed normal caged activity until the time of study.

C. Histologic Test Procedure.

The rat was anesthetized and placed on an operating board, following cast removal if applicable. A skin incision was made on the medial aspect of the knee, on the left utilizing the old incision. Using the dissecting microscope the medial collateral ligament was exposed, mobilized and then freed from its femoral and tibial insertions using sharp dissection. The repair site of the ligament was not subject to stress during the dissection and all tissues were kept moist with an isotonic saline solution. The ligament was immediately placed in Karnovsky's fixative at room temperature for 30 minutes. The procedure was repeated for the right knee and then the rat was sacrificed with an overdose of Sodium Pentobarbitol. After thirty minutes of fixation, the ligament was trimmed into smaller segments for study. The area bounded by the sutures were used for study and a corresponding area of the right medial collateral ligament was likewise trimmed. The ligaments were then fixed for a further two hours, washed in sodium cocadylate buffer and then placed in this buffer until they were embedded in pure epon moulds.

From these epon moulds, 1.5 micron thin sections were cut and stained with Toluidine blue for light microscopy. After evaluation under the light microscope, the ligament was trimmed further and 600-900Å thick sections were cut from appropriate areas. These were stained with saturated uranyl acetate

and lead citrate for electron microscopy.

D. Histologic Evaluation.

1. Light Microscopy

In order to objectively evaluate the maturity of a healing ligament, a Maturity Index Score and a Maturity Index Scale were devised. These are based on an evaluation of the repairing area of the ligament using the light microscope and looking at four variables:

a) The numbers of fibroblasts per high power field (h.p.f.). Three separate representative areas on two separate sections of the area of ligament repair were looked at, at 400X magnification and the numbers of fibroblast nuclei were counted. This number was averaged and a range was determined.

b) Orientation of the fibroblasts in relation to the longitudinal ligament axis. That is whether they were parallel to this axis or whether they were angled to it or whether there was any organization at all to their alignment.

c) Orientation of the collagen fiber bundles to the longitudinal ligament axis. Branching was defined as fibers which angled from the normal axis into a second plane. Transverse fibers and fibers seen in cross-section were also noted.

d) Amount of collagen present. This was graded on whether the collagen was tightly packed or loosely packed or mixed.

The numerical assignment for each variable is listed in Table I with the lowest numbers indicating full maturity.

These variables are then totaled and applied to the Maturity Index Scale (Table II) which represents phases of ligament healing following the inflammatory phase and continuing to full maturity. The remodelling phase, which lasts the longest period of time, has been divided into three stages:

early, mid and late.

This scale then enables investigators to numerically compare ligament healing throughout its total process and in fact should apply to all species.

2. Electron Microscopy

Factors studied with the electron microscope included the numbers and orientation of the fibroblasts as well as the size of the cells and the maturity or level of metabolic activity of the fibroblasts. The amounts of rough endoplasmic reticulum, golgi complexes and mitochondria were taken as an indication of this activity with the most immature fibroblasts having the greatest amounts of these intracellular organelles as well as being the largest in size. The collagen fibers were also looked at in terms of their organization, size and amount present.

E. Biomechanical Test Procedure.

The rat was anesthetized and killed with an overdose of Sodium Pentobarbitol. Both hind limbs were then removed and dissected free of all soft tissue except for the ligaments and capsule of the knee joint. Tissues were kept moist at all times with an isotonic saline solution. The limbs were then placed in specially designed holders and pre-set in rapid setting epoxy with the knee at 90 degrees of flexion (Fig. 2). This angle was chosen based on a previous study by Tipton[34] where he radiologically studied the physiologic angle of knee flexion in the rat and found it to be 87.7 ± 4.7 degrees. This holder had a removable bar on it to stabilize the preparation thereby protecting the ligament from stresses until the time of test. Once the epoxy had set and just prior to testing, the knee was then further dissected using a dissecting microscope. All capsular attachments and ligaments were removed except for the medial collateral ligament. This provided a functional unit of a femur-medial collateral ligament-tibia preparation. The holders were then

placed in an Instron materials testing machine (Fig. 3), the stabilizer bar was removed, and the ligament was tested until failure with the force elongation curve being recorded on an oscillograph. The rate of extension chosen was 0.02 mm/second. This is a slower rate than that causing most injuries, however, in a previous study by Tipton[34], he found that there was no significant difference in this rate versus a faster physiologic rate of loading when applied to the medial collateral ligament of the rat.

All ligaments were studied within three hours of sacrifice. Tipton[34] studied the influence of delay in testing on the maximum load at failure and he found that there was no significant difference between immediate testing and a delay of eight hours. Longer than eight hours seemed to produce a difference in values.

F. Biomechanical Evaluation.

The oscillograph recordings or force elongation curves were analyzed for the following variables:

1. Maximum load in kilogram force at the time of failure.
2. Separation Force Ratio (S.F.R.). This is the ratio of the maximum load at failure in kilograms to the body weight of the animal in kilograms. Tipton[34] has shown that the maximum load at failure of the rat medial collateral ligament, varies with the body weight of the rat and that by dividing the force by the body weight, a constant S.F.R. is obtained. This has also been found to be a constant ratio in other species[20].
3. Elongation of the ligament in millimetres prior to rupture. This was determined by calculating the time until failure off of the graph and then multiplying by the rate of extension. As all of our rats in their respective groups were of similar weights and size and we did not have a way of measuring the total length of the ligament accurately to very small

differences, for comparison purposes, the elongation was considered to be an indication of the strain of the ligament (change in length divided by the original length). The original resting length measured by our methods was 9.7 to 9.9 milimeters in all of the rats studied. The amount of elongation of the bone and holders was taken to be insignificant.

4. Compliance is the ratio of the elongation in milimeters to the load at failure or separation force in kilograms. This is essentially the inverse of Youngs Module of Elasticity.

Tensile strength or stress (force per unit area) could not be calculated as we did not have an accurate method of measuring the cross-sectional area of a ligament the size of a rat medial collateral ligament.

Statistical analyses of the biomechanical data were performed with a computor using, where appropriate, students t-test and paired t-test, analysis of variance and multiple range test. Values were defined to be statistically significant at P values less than 0.05.

In addition to this evaluation of the force elongation curve, the site of ligament failure was also noted and the range of motion of the knee was measured at the time of testing.

RESULTS

A. General.

All wounds healed primarily with no evidence of infection. Any rats developing complications which consisted of pin tract infections, were discarded from the study. A total of 61 rats were studied, 39 biomechanically and 22 histologically. All rats continued to gain weight although while a rat had one limb immobilized its rate of weight gain was decreased. All limbs immobilized, demonstrated gross muscle atrophy which appeared to increase as the length of time immobilized increased although this was not quantitated.

A normal rat knee has approximately 115 degrees of motion subscribing an arc of between 45 and 160 degrees. When compared to the control knee, all immobilized knees showed a decrease in this range of motion (See Table III). In Group B (2 weeks immobilization), a decrease to 85 to 95 degrees of motion was seen. This had been regained from the sixth week test onwards. In Group C (6 weeks immobilization), a decrease to 20 to 40 degrees of motion was seen. At twenty weeks, this group still lacked 20 to 30 degrees of motion. In Group D (10 weeks immobilization) a decrease to only 20 to 30 degrees of motion was seen. At twenty weeks post-operative, this group still lacked 40 to 60 degrees of motion. Group A had a normal range of motion at all test periods.

B. Histology - Light Microscopy.

1. Controls (Figs. 4 and 5).

Control ligaments displayed a regularity of collagen fiber bundles within the ligament which were longitudinally oriented and densely packed.

Fibroblasts were small and very elongated in appearance with small darkly staining nuclei. The number of fibroblasts per high power field was 3-6. The Maturity Index Score was four.

2. Two Weeks.

A large amount of callous was seen which appeared greater in Group A. Microscopically the site of laceration was easily identified in both groups although grossly they appeared healed. Occasional inflammatory cells were seen in both groups. In Group A (no immobilization), (Figs. 6 and 7), there was a marked hypercellularity of over 100 fibroblasts per high power field. These fibroblasts were larger in size and had large pale staining nuclei. The fibroblasts displayed some longitudinal orientation along the ligament axis. There was early collagen fiber formation in a longitudinal direction.

In Group B (2 weeks immobilization), (Figs. 8 and 9), there was also an increase in the number of fibroblasts to 30-45 per high power field. These fibroblasts were also larger in size and had large pale staining nuclei although this difference was not as marked as in Group A. Early collagen fiber formation was present but this displayed no organization.

The Maturity Index Score for Group A was 13 and for Group B it was 14.

3. Six Weeks.

All ligaments appeared grossly healed. There were no inflammatory cells seen. All ligaments displayed some callous although this was less than at two weeks.

Group A (Figs. 10 and 11), showed a decrease in the numbers of fibroblasts to 32-43 per high power field. The fibroblasts were smaller in size and had smaller darkly staining nuclei. These fibroblasts were arranged longitudinally, in alignment with the ligament axis. The collagen fibers were longitudinally oriented completely bridging the lacerated area and were as

densely packed as in controls. The Maturity Index Score was Eight.

Group B (Figs. 12 and 13), showed a smaller decrease in the numbers of fibroblasts to 18-31 per high power field. These fibroblasts were also smaller with smaller darker staining nuclei. They were now mainly longitudinal in orientation with the ligament axis. Collagen fibers were now present in increased amounts and these appeared to be more longitudinal and better organized than at two weeks. However they still displayed branching and were not as densely packed or as well organized as in Group A. The Maturity Index Score was eleven.

In Group C (Figs. 14 and 15), there was an increase in the fibroblasts to 52-64 per high power field. The fibroblasts were larger than in Groups A and B with large darkly staining nuclei and with large numbers of intracellular inclusions. Their arrangement tended to be irregular and not in alignment with the ligament axis. The collagen fiber bundles tended to be irregular, with branching and fibers at right angles to each other indicating little organization. They were also less densely packed. The Maturity Index Score was fourteen.

4. Ten Weeks.

All ligaments appeared grossly healed and callous formation was minimal.

In Group A (Figs. 16 and 17), there was a further decrease in the numbers of fibroblasts to 18-28 per high power field. These fibroblasts were small with small darkly staining nuclei. The fibroblasts were all oriented longitudinally. Collagen fiber bundles were longitudinally oriented, densely packed and well organized appearing the same as controls. The Maturity Index Score was five.

Group B (Figs. 18 and 19), showed a decrease in the numbers of fibroblasts to 15-25 per high power field. The fibroblasts were small with small

darkly staining nuclei. Occasional more immature looking cells were seen. The collagen fiber bundles were longitudinally oriented, regular and densely packed. The Maturity Index Score was 5.

In Group C (Figs. 20 and 21), the numbers of fibroblasts had decreased to 16-28 per high power field and the fibroblasts were more longitudinally oriented although some disarray was still present. The fibroblasts were also smaller in size and had smaller nuclei. The collagen fiber bundles were more longitudinally oriented although there was still a large amount of branching from the ligament axis. The collagen fibers were not as densely packed as in Groups A and B. The Maturity Index Score was nine.

In Group D (Figs. 22 and 23), the numbers of fibroblasts present was 45-52 per high power field. These fibroblasts were larger than in the other groups and displayed an irregular organization which was in places at right angles to the ligament axis. The collagen fiber bundles were also present in a disorganized fashion with branching and fibers at right angles to the ligament axis. They were also not as densely packed as in the other groups. The Maturity Index Score was twelve.

5. Twenty Weeks.

All groups were grossly healed with only minimal callous present in Group D.

In Group A (Fig. 24), the numbers of fibroblasts had decreased to 4-8 per high power field. The fibroblasts were the same as controls in terms of cellular and nuclear size as well as longitudinal organization. The collagen fiber bundles were also the same as controls with a densely packed, regular, longitudinal arrangement. The Maturity Index Score was four.

In Group B (Figs. 25 and 26), the numbers of fibroblasts had decreased to 10-15 per high power field. The fibroblasts appeared the same as controls

in terms of cellular and nuclear size with a longitudinal orientation. The collagen fiber bundles were longitudinally oriented and densely packed, similar to controls. The Maturity Index Score was five.

In Group C (Figs. 27 and 28), the number of fibroblasts was still 14-22 per high power field but they were now longitudinally oriented with only a small number being angled to the ligament axis. The fibroblasts had decreased in size. The collagen fibers were densely packed and regular with mainly a longitudinal orientation but with some branching. The Maturity Index Score was seven.

In Group D (Figs. 24 and 30), the numbers of fibroblasts had decreased to 20-30 per high power field and these fibroblasts had decreased in size to that of the controls. The fibroblasts were mainly longitudinal in orientation with some angled to the ligament axis. The collagen fiber bundles were densely packed and were arranged in a longitudinal fashion with some branching present. The Maturity Index Score was seven.

The results of Maturity Index Score applied to the Maturing Index Scale, are listed in Table IV and depicted graphically in Figure 31. This illustrates the rapid maturation of Group A (no immobilization) which achieved full maturity at twenty weeks post-operative. The other groups all displayed a retarded rate of maturation while the limb was immobilized. This increased once mobilization commenced but none of these groups had achieved full maturity at twenty weeks post-operative and appeared to be plateauing in the late remodelling phase.

C. Histology - Electron Microscopy.

1. Control (Figs. 32,33,34).

The fibroblasts displayed evidence of maturity and minimal metabolic activity with only small numbers of mitochondria, rough endoplasmic reticulum

and golgi complexes. The collagen fibers demonstrated the regular 640Å banding characteristic of collagen and were densely packed and oriented in a longitudinal fashion along with the fibroblasts.

2. Two Weeks.

In Group A (Figs. 35 and 36), the fibroblasts were markedly increased in numbers. The fibroblasts were large and demonstrated a high level of cellular metabolic activity. There were large numbers of mitochondria, abundant rough endoplasmic reticulum, large golgi complexes and a large number of vesicles. The collagen fibers demonstrated the regular 640Å periodicity of banding and displayed some longitudinal organization. These fibers were small in diameter and loosely packed. A small amount of extracellular debris was present.

The fibroblasts in Group B (Figs. 37 and 38), were not as large, nor were they as active metabolically. They had increased numbers of mitochondria and rough endoplasmic reticulum although not as much as in Group A. There was no regularity to the orientation of the fibroblast. The collagen fibers were smaller in diameter, very loosely arrayed and displayed no organization with fibers seen running in all three planes. The maturity and metabolic activity were not as well developed as in Group A, and there was a large amount of extracellular debris still present.

3. Six Weeks.

In Group A (Figs. 39 and 40), the fibroblasts had decreased in size, numbers, and level of metabolic activity. Increased numbers of mitochondria and rough endoplasmic reticulum were still present, but this was decreased from at two weeks. The collagen fibers were larger in size, densely packed and organized mainly longitudinally.

Group B (Figs. 41 and 42) fibroblasts were similar in size, numbers and

level of metabolic activity to Group A and had attained some longitudinal organization. The collagen fibers displayed the characteristic 640Å banding and had increased in size although a large degree of variability was present. The orientation of the collagen was longitudinal with branching and transverse fibers but displaying some organization. Extracellular debris was still present but only in small amounts.

Group C (Figs. 43 and 44) fibroblasts were larger in size than Groups A or B and were very active metabolically with abundant mitochondria, rough endoplasmic reticulum and golgi complexes. The collagen fibers were smaller in size, loosely packed in some areas and were poorly organized with fibers seen running in three different planes. There were large amounts of extracellular debris.

4. Ten Weeks.

The fibroblasts of Group A (Figs. 45 and 46), showed a further decrease in numbers, size and level of metabolic activity. The amount of mitochondria, rough endoplasmic reticulum and golgi complexes was still greater than in controls. The collagen fibers were larger in diameter, densely packed and mainly longitudinal in organization.

Group B (Figs. 47 and 48) fibroblasts were larger than in Group A but had also decreased both in size in the level of metabolic activity. The collagen fibers were regular in size, were densely packed and were longitudinally oriented but with some branching.

Group C (Figs. 49 and 50) fibroblasts were larger than in Groups A or B and displayed greater metabolic activity than these groups with increased amounts of rough endoplasmic reticulum, mitochondria and golgi complexes. The collagen fibers were more densely packed than at six weeks and were better organized. There still were fibers running in all three planes in close prox-

imity and extracellular debris was still present.

Group D (Figs. 51 and 52) fibroblasts were not as large as in Group C nor were they as metabolically active with fewer numbers of mitochondria, rough endoplasmic reticulum, and golgi complexes. The cells displayed no definite longitudinal organization. The collagen fibers were not as densely packed as in the other groups and they were smaller in diameter. The collagen displayed little organization with fibers running in all three planes. Extracellular debris was present in increased amounts.

5. Twenty Weeks.

Group A (Figs. 53 and 54) fibroblasts were identical to controls in size and level of metabolic activity. The collagen fibers were longitudinally oriented and as densely packed as in controls. The collagen fiber diameter was similar to controls although some smaller fibers were still seen.

Group B (Figs. 55 and 56) fibroblasts were also similar to controls in both size and metabolic activity with decreased amounts of rough endoplasmic reticulum and golgi complexes from at ten weeks. The collagen fibers were longitudinally oriented and densely packed although not as dense as in controls. Occasional branching was seen and the collagen fiber diameter was less than that of Group A or controls.

Group C (Figs. 57 and 58) fibroblasts were still more numerous, larger and more metabolically active than controls although the amount of rough endoplasmic reticulum and golgi complexes had decreased from at ten weeks. The organization of the collagen fibers had improved although branching was still quite extensive and occasional groups of fibers were seen in a cross section beside longitudinal ones. The collagen fibers were more densely packed than at ten weeks but still less than controls and the diameter of the fibers was also still smaller than in controls.

Group D (Figs. 59 and 60) fibroblasts were more numerous than in controls. Their level of metabolic activity was less than that of Group C but not the same as controls. The numbers of mitochondria and rough endoplasmic reticulum had decreased. The collagen fibers displayed minimal organization with an overall longitudinal array but with a large degree of branching fibers at right angles and fibers in cross-section. They were also not as densely packed or as large in diameter as in Group A or in controls.

D. Biomechanical Testing.

Just prior to testing, the ligaments were inspected grossly with the dissecting microscope (40X) and the findings agreed with those reported in the previous section on Histology in terms of appearance of healing and callous formation.

1. Maximum Load at Failure.

See Table V where the results are listed. This will not be discussed as results correlate with the Separation Force Ratio results which will be discussed in detail.

2. Separation Force Ratio (S.F.R.).

As shown by Tipton[34], the S.F.R., which is a ratio of the separation force or maximum load at failure of the ligament to the animal body weight, is an accurate method of comparing results within an animal experimental model. Table V lists the S.F.R. of all of the ligaments tested and these are displayed graphically in Figure 61.

A sharp rise is seen in the first two weeks in Groups A and B with Group A showing the greatest increase. Applying the t-test to these results, $p = 0.056$, a value not quite significant, but one that probably would have been if our population in these groups was larger. At six weeks, Groups A and B were significantly different from Group C ($P < 0.0005$). At ten weeks,

Groups A and B are showing a fall off in their rate of increase while Group C, now out of its cast for four weeks, showed a sharp increase. At twenty weeks, Groups A, B and C are levelling off while Group D has shown more of an increase now that they were out of their casts. At this time there is a significant difference between these groups ($p = 0.0005$).

The S.F.R. of the repaired ligament was compared to the S.F.R. of the control ligament as shown in Table VI and illustrated graphically in Figures 62 and 63. These results parallel those discussed above. At two weeks post-operative, Group A was 37% stronger than Group B and had achieved a strength which was 46% of control strength while Group B was only 29% of control strength. Applying the t-test $p = 0.055$, a value not quite significant but one that probably would have been if our populations in these groups were larger. At six weeks post-operative, Group A was 65% of controls, Group B was 56% of controls and Group C was 39% of controls. These differences were significant ($p = 0.0004$). Group A was 96% stronger than Group C at this time. At ten weeks post-operative, Group A was 87% of controls, Group B was 64% of controls, Group C was 51% of controls and Group D was 37% of controls. The population of these groups tested at this time period, was too small to apply any meaningful statistical analysis. At twenty weeks post-operative, Group A was now 83% of controls, Group B was 71% of controls, Group C was 66% of controls and Group D was 48% of controls. These differences were significant ($p = 0.0005$). At this time, Group A was 11% stronger than Group B, 32% stronger than Group C and 71% stronger than Group D. All groups displayed a significant change over the time studied between their values at two weeks, six weeks and twenty weeks when studied within the individual groups ($p < 0.01$).

This analysis showed that Group A (no immobilization) had a significantly higher S.F.R. than the other groups as compared to controls and

regained its functional unit strength at a faster rate than the other groups. Group B (two weeks immobilization) also displayed a significantly higher S.F.R. than Group C at six weeks but this difference diminished over the next fourteen weeks. It was also seen that once the casts were removed and mobilization commenced, the rate of gain of strength increased.

3. Elongation.

See Table V where the results are listed and Figure 64 where they are displayed graphically. The results do not indicate any specific pattern nor are the differences within groups significant. The elongation of the repaired ligament was compared to the elongation of the control ligament as listed in Table VI and illustrated in Figure 65. This shows that all groups follow a similar pattern with the maximum elongation occuring at 6 weeks (98% - 117%) then steadily decreasing to 20 weeks where values were 61% - 72% of controls. When statistical analysis was applied, the differences between the groups were not significant nor were the differences within the individual groups at the various time intervals significant ($p = 0.087 - 0.34$).

4. Compliance.

Compliance is the ratio of the amount of elongation of the ligament to the unit load at failure. The results are listed in Table V and illustrated graphically in Figure 66. These results show a trend towards an improvement in the compliance in all groups over time without a significant difference between the groups at the various time periods. The compliance of the repaired ligament was compared with the control ligament as listed in Table VI and illustrated in Figure 67. This shows an improvement in compliance over time with no statistical differences in the groups at the various time periods. When looking at individual groups over time, both Groups A and B showed a significant improvement over time ($p < 0.01$). However the differ-

ences within Groups C and D were not significant. At 20 weeks, all groups had attained a compliance which was not statistically different from controls whereas when compared to controls all groups had previously been statistically different ($p < 0.05$).

5. Site of Ligament Failure.

This data is listed in Tables VII and VIII and shows that from six weeks onwards, all failures occurred at a site other than at the repair site indicating that by this time the strength of the repair was greater than that of the ligament itself or of the ligament-bone insertion. At two weeks, one of the specimens from Group A failed at the tibial insertion, indicating that it had already achieved a strength of repair greater than the ligament-bone insertion. As can be seen, 83% of the failures occurred at the tibial insertion. This concurs with the results of Tipton [34] and Zuckerman[38] and supported by Noyes[26]. Noyes showed that the tibial insertion of the medial collateral ligament was directly into bone and periostium and that with immobilization of 8 weeks, this area underwent subperiosteal resorption actually disrupting the attachment of the ligament in places, leaving it attached only to periostium. This also explains why this appears to be the weakest point in the normal medial collateral ligament. Laros [20] and associates have shown that the tibial insertion of the medial collateral ligament is subject to resorptive changes even in non-immobilized limbs where the animal has been caged and this led to an area of weakness definable histologically.

DISCUSSION

A. The Experimental Model.

Ligament and tendon healing has been studied in various animal experimental models including the rat[7,11,15,17,18,19,21,22,27,28,29] and the manner of healing is identical in all of these models, as has been outlined. These studies have been histologic, biochemical, and or, biomechanical but up until now, an objective method of histologically assessing and comparing ligament healing has not been reported.

The effects of immobilization on normal ligaments have also been studied in various animal models[4,10,20,23-26,34-36,38]. However the effects of varying periods of immobilization on ligament healing have not been previously studied. The immobilization method utilized in this study combined methods used in other studies[7,10,18,20,26-29,35] and proved to be very satisfactory with minimal complications.

Using the rat as the model had advantages which included 1) the rats used were a pure inbred strain of similar size and maturity which decreases the variables in the experimental model and provides far greater reproducibility of the experiments, 2) the rats were easy to work with and perform the experiments on as well as being an inexpensive animal to maintain, 3) the anatomy of the rat knee is similar to other animals and humans with the only significant differences being the presence of ossified menisci in the adult rat and the habitually flexed position of the rat knee.

B. Histology.

The Maturity Index Score and Maturity Index Scale form a simple and

easily applied method of objectively assessing the stage of maturity of a repairing ligament allowing numerical comparison betwen various groups. It takes into account the factors which appear to be the most important in assessing the healing process of a ligament. That is, the numbers and orientation of the fibroblasts and the amount and orientation of the collagen fiber bundles. This data has shown that the maturation of the ligament progressed more rapidly in the group with no immobilization, and at twenty weeks it had attained the maturity of control ligaments. It also shows that the maturation process is slow while the limb is immobilized and then progresses at a more rapid rate once the cast is removed and the leg mobilized. All immobilized groups displayed this trend and none of the immobilized groups had fully matured at twenty weeks post-operative.

This scoring system has been supported by the electron microscopic work which has demonstrated the metabolic activity of the fibroblasts at the various stages of healing showing that the metabolic activity decreasd with increasing maturity. Also shown was that the greatest level of metabolic activity was present at cast removal and with increasing periods of mobilization, this initial level of metabolic activity decreased. The fibroblasts that displayed the greatest level of metabolic activity were those seen at two weeks in the group which had no immobilization. Also demonstrated, was the maturation of the collagen fibers which progressed more rapidly in the group with no immobilization and in the other groups once mobilization commenced. At two weeks, organized collagen formation was already seen in Group A. The collagen maturation progressed with increased deposition of collagen fibers which gradually increased in size and longitudinal parallel organization with mobilization. With immobilization, the collagen appeared to be laid down in a randomized fashion and with mobilization, this collagen appeared to become or-

ganized and mature.

The other interesting factor, was the amount of extracellular debris present. In Group A, this was minimal at two weeks and nonexistant after this. In the other groups extracellular debris was present for quite a long period following this. In Group D, there was still debris present at 10 weeks post-operative. It appeared that this debris was rapidly cleared once mobilization commenced.

Therefore, with the application of physiologic stresses and mobilization of the extremity, the healing ligament histologically matured more rapidly with: 1) earlier maturation of fibroblasts which had been more active metabolically, 2) earlier alignment of the fibroblasts along the longitudinal ligament axis, 3) earlier alignment of the collagen fibers along the longitudinal ligament axis 4) increased deposition of larger more mature collagen 5) increased clearing of extracellular debris.

These factors were shown to be in progress at as early as two weeks post-operative in the group in which mobilization had begun immediately.

C. Biomechanical Testing.

The results of the biomechanical testing correlated with those derived histologically. It showed that there was a more rapid and significantly greater increase in the strength of the ligament-bone unit with no immobilization. At two weeks post-operative, one of the ligaments from Group A, had already attained a strength of repair which was greater than the strength of the tibial insertion, indicating a more advanced state of maturation.

After six weeks post-operative, our results paralleled those in the literature which shows that with increased immobilization of a limb, the strength of the ligament and of the ligament-bone unit decreases[26,27]. With increased immobilization, there is also a longer period before the ligament

strength returns to normal[26]. This data also indicates that once the healing ligament has achieved a certain level of maturation, the weakest point in the medial collateral ligament is its tibial insertion and not the repair site. Since all groups tested at this time were at different stages of maturation (Maturity Index Scale: Group A-3, Group B-4, Group C-5), this supports the concept that mobilization should begin early as there was greater strength, even in the most immature, in the repair site than in the tibial insertion.

The compliance of the healing ligament was significantly increased at the time of cast removal and with mobilization, this rapidly decreased to that of the control groups. This indicates that with mobilization, the ligament experienced a more rapid maturation and stiffening of the repair such that the application of a load led to less elongation or stretch of the ligament.

We had no way of accurately judging the stability of the knee joints of the rats to varus or valgus stresses, except for gross palpation of the knee joint when subjected to these stresses. With this gross method of testing, no instability was detected. This is an important factor to consider when evaluating functional properties of a healing ligament and should probably be included in future studies of this nature. It is unlikely, in our model, that any significant laxity of the healing ligament occurred as the rats did not take part in any stressfull activities and tended to splint the operated limb until they were comfortable. They were seen in all cases, to be easily moving about their cages with full weight bearing in no apparent discomfort one to three days post-operative. Knee motion was present in the group with no immobilization from the first post-operative day onwards. Therefore, we feel that only physiologic stresses were applied to this healing ligament.

The leg wasting and decreased range of motion seen in all of our rats

that were immobilized parallels that reported in other series[10,26,36]. It shows the dramatic decrease in the range of motion of the knee that occurs after prolonged immobilization and the length of time it takes to regain this motion. At twenty weeks post-operative neither Group C (6 weeks immobilization) or Group D (10 weeks immobilization) had regained their full range of motion.

D. Clinical Relevance.

George Perkins[30] stated: "If orthodox views are correct, they will prevail against any opposition, if they are false, the sooner they are discarded the better. The proposition I am putting forward, is that movement is often better treatment than rest". He felt that inflammation should be treated by rest as long as one interpreted inflammation correctly as being a time when the body reacts to a harmful insult. Once a ligament has been repaired this insult then ceases and so does inflammation although the inflammatory response slowly subsides. Following this, is the repair stage where the debris is removed and the repair tissue laid down. According to Mason and Allen[22], the inflammatory phase ends on the third to fifth day. Perkins[30] then feels that the repair phase should "be treated by activity, namely the resumption of function and movement".

Ernst Dehne and Richard Torp[9] feel that joint injuries including ligamentous injury, should be treated by immediate mobilization. This is based upon the "Spinal adaption concept", where they feel that repairs are "an adaptive process sensitive to adverse external stimuli and responsive to regulated feed-back exposure".

Salter[32,33] has shown, in his experiments on continuous passive motion used following intra-articular fractures in rabbit knees which were surgically repaired, that passive motion begun immediately, was beneficial to

the healing process of the articular cartilage. He has shown that continuous passive motion is well tolerated in the rabbit and when applied to intra articular fracture of the knee joint, there was:

" 1) No adhesion formation

2) significantly faster reconstitution of the subchondral bone plate

3) significantly more frequent healing of the articular cartilage defects

4) significantly less development of degenerative arthritis post-op."

Haggmark and Erikson[14] presented a prospective randomized trial of sixteen patients where a standard cylinder cast was compared with a mobile cast brace with forty degrees of motion, both applied seven days post operative following anterior cruciate ligament reconstruction. They evaluated muscle atrophy using muscle biopsies and biochemical and histologic methods. Results were also assessed clinically up to twelve months post-operative. They found no difference in the functional and clinical end results of the two groups while the group with the standard cast showed atrophy of type I (slow twitch) fibers, not seen in the cast-brace group. They also noted a more rapid return of a normal range of motion (eight weeks versus sixteen weeks) and a more rapid return to sports in the cast-brace group. They feel that the cast-brace protected the reconstructed ligament from valgus, varus and rotational stresses while allowing a limited range of motion. This allows physiologic stresses to be applied to the healing ligament and provides the intra-articular benefits of joint motion. It also allows for increased circulation to the extremity and minimizes muscle wasting.

Our results are in agreement with the views just presented. We feel

that early mobilization of the joint and extremity with the application of physiologic stresses to the healing ligament is beneficial to the healing process and maturation and to the function of the extremity as a whole. The method of Haggmark and Erikson[14] is probably the method of choice for clinical use as it provides some protection from abnormal stresses but allows for joint motion and the application of physiologic stresses. It also is applied early as soon as swelling and pain will allow.

The use of no cast immobilization following knee ligament surgery is an interesting concept to consider for clinical use. Experimental work being done with continuous passive motion may support this concept, but for now, most investigators believe that some form of post-operative protection is required[4,5,14,26-29].

SUMMARY AND CONCLUSION

The effects of varying periods of immobilization on the maturation and strength of the healing process of the rat medial collateral ligament were studied. A Maturity Index Score and a Maturity Index Scale were devised based on:

1) the numbers of fibroblasts present
2) the orientation of the fibroblasts
3) the orientation of the collagen fiber bundles
4) the amount of collagen present

Utilizing this scale, supported by the electron microscopic study, it was shown that a healing ligament matures more rapidly when exposed to physiologic stresses and mobilization of the extremity, and that immobilization delays this maturation.

This has been correlated with biomechanical testing which showed a more rapid return of the strength of the ligament and ligament-bone unit with mobilization of the extremity without displaying any detrimental effects to the elongation or compliance of the ligament. The detrimental effects of immobilization on ligament-bone insertion strength were shown as well as the decrease in the range of motion of the knee.

We feel that clinical studies and further animal studies are warranted to document the clinical application of immediate or early mobilization of extremeties following acute ligament repairs of the knee.

Maturity Index Score

			Score
1.	Numbers of Fibroblasts per high power field		
	Normal ligament	<10	1
		10-30	2
		30-50	3
		50-100	4
	Immature and Active in Repair	>100	5
2.	Orientation of the Fibroblasts.		
	Longitudinal in alignment with the ligament axis		1
	longitudinal plus angled to the ligament axis		2
	random order including transverse to the axis		3
3.	Orientation of the collagen fiber bundles		
	Longitudinal in alignment with the ligament axis		1
	mainly longitudinal with some fibers angled (Branching)		2
	longitudinal with extensive branching		3
	minimal organization with some transverse fibers		4
	random organization in all 3 planes		5
4.	Amount of Collagen Present.		
	tightly packed		1
	some areas tight, some loose		2
	loosely packed		3

TABLE I

Maturity Index Scale

Group		Maturity Index Score
1	Normal - mature	4
2	Late remodelling phase	5 - 7
3	Mid remodelling phase	8 - 10
4	Early remodelling phase	11 - 13
5	Immature - in proliferative phase	14 - 16

TABLE II

Range of Motion

Group	Total motion	
Control	115^{o}	$45\text{-}160^{o}$ arc
A	115^{o}	at 2 wks and 20 wks
B	$85^{o}\text{-}95^{o}$	at 2 wks
	115^{o}	at 6 wks and 20 wks
C	$20^{o}\text{-}40^{o}$	at 6 wks
	$85^{o}\text{-}95^{o}$	at 20 wks
D	$20^{o}\text{-}30^{o}$	at 10 wks
	$55^{o}\text{-}75^{o}$	at 20 wks

Table III

Maturity Index Score

Group	Time in Wks	Nos. of Fibroblasts per h.p.f.	Orientation of Fibroblasts	Orientation of Collagen	Amount of Collagen	Total	Maturity Index Scale
A	2	5	2	3	3	13	4
B	2	3	3	5	3	14	5
A	6	3	2	2	2	9	3
B	6	2	2	4	3	11	4
C	6	4	3	5	2	14	5
A	10	2	1	1	1	5	2
B	10	2	1	1	1	5	2
C	10	2	2	3	2	9	3
D	10	3	3	4	2	12	4
A	20	1	1	1	1	4	1
B	20	2	1	1	1	5	2
C	20	2	2	2	1	7	2
D	20	2	2	2	1	7	2
Control		1	1	1	1	4	1

See text for description of Maturity Index Score and Maturity Index Scale.

TABLE IV

Biomechanical Testing of Medial Collateral Ligament

Group	Time Wks	N	Maximum Load kg. Left	Maximum Load kg. Right	S.F.R. Left	S.F.R. Right	Elongation mm Left	Elongation mm Right	Compliance mm/kg Left	Compliance mm/kg Right
A	2	2	1.30 ±.11	2.80 ±.02	2.73 ±.19	5.90 ±.05	4.32 ±.26	3.94 ±.13	3.34 ±.10	1.41 ±.06
B	2	2	0.93 ±.02	3.25 ±.48	2.00 ±.13	6.92 ±.72	3.30 ±.51	3.94 ±.38	3.54 ±.47	1.23 ±.07
A	6	4	2.38 ±.36	3.67 ±.30	4.70 ±.68	7.27 ±.59	3.49 ±.58	3.11 ±.79	1.47 ±.18	0.84 ±.15
B	6	3	1.93 ±.27	3.47 ±.50	4.13 ±.55	7.45 ±1.00	3.30 ±.36	3.38 ±.52	1.75 ±.36	1.00 ±.24
C	6	6	1.09 ±.21	2.78 ±.30	2.40 ±.45	6.13 ±.61	3.28 ±.59	3.40 ±.70	3.26 ±.15	1.21 ±.17
A	10	1	2.75	3.14	5.15	5.87	3.56	3.81	1.30	1.21
B	10	1	2.45	3.86	4.82	7.59	2.54	3.30	1.04	0.86
C	10	1	2.14	4.18	4.19	8.18	2.03	2.29	0.95	0.55
D	10	2	0.99 ±.04	2.73 ±.14	2.12 ±.50	5.80 ±.06	2.29 ±.26	2.80 ±.26	2.48 ±.78	1.02 ±.06
A	20	3	3.29 ±.11	3.97 ±.06	5.76 ±.32	6.93 ±.19	2.88 ±.48	4.06 ±.55	0.89 ±.20	1.02 ±.13
B	20	3	2.96 ±.15	4.18 ±.02	5.21 ±.52	7.34 ±.31	3.30 ±.62	5.50 ±.43	1.13 ±.25	1.32 ±.14
C	20	4	2.56 ±.14	3.89 ±.14	4.36 ±.25	6.62 ±.23	3.43 ±.56	4.89 ±1.10	1.35 ±.25	1.27 ±.30
D	20	4	1.98 ±.26	4.16 ±.14	3.37 ±.41	7.09 ±.33	2.61 ±.60	3.75 ±.45	1.38 ±.46	0.90 ±.11
Z	0	3	0.16 ±.02	2.82 ±.50	0.32 ±.05	5.83 ±1.01	3.13 ±.54	3.94 ±.52	27.0 ±5.2	1.40 ±0.05

Group A=No Immobilization; Group B=2 wks Immobilization; Group C=6 wks Immobilization; Group D=10 wks Immobilization; Group Z=test at time of surgery. Table lists mean values in the Left (repaired) and the Right (control) Legs.

Table V

Repaired Ligament/Control Ligament

Group	Time Wks	N	Maximum Load	S.F.R.	Elongation	Compliance
A	2	2	0.46	0.46	1.10	2.37
B	2	2	0.29	0.29	0.86	2.88
A	6	4	0.65	0.65	1.17	1.82
B	6	3	0.56	0.56	0.98	1.77
C	6	6	0.39	0.39	1.05	2.69
A	10	1	0.88	0.88	0.93	1.07
B	10	1	0.64	0.64	0.77	1.21
C	10	1	0.51	0.51	0.89	1.73
D	10	2	0.36	0.36	0.82	3.02
A	20	3	0.83	0.83	0.72	0.88
B	20	3	0.71	0.71	0.61	0.88
C	20	4	0.66	0.66	0.75	1.15
D	20	4	0.48	0.48	0.72	1.60
Z	0	3	0.06	0.06	0.79	19.14

Group A=No Immobilization; Group B=2 wks Immobilization; Group C= 6 wks. Immobilization; Group D=10 wks Immobilization; Group Z=test at time of surgery. Table lists mean values of the ratio of the Left (repaired) Ligament to the Right (control) Ligament.

TABLE VI

		Left	Site of Failure				Right				
Group	Time wks	N	Tibial avulsion	Femoral avulsion	Midsubstance tear at sutures	Midsubstance tear not at sutures	Tibial avulsion	Femoral avulsion	Midsubstance tear at sutures	Midsubstance tear not at sutures	N
Z	0	3			3		3				3
A	2	2	1		1		2				2
B	2	2			2		2				2
A	6	4	4				4				4
B	6	3	3				2			1	3
C	6	6	4	1		1	6				6
A	10	1	1				1				1
B	10	1	1				1				1
C	10	1	1				1				1
D	10	2	2				2				2
A	20	3	3				3				3
B	20	3	2	1			3				3
C	20	4	3	1			4				4
D	20	4	2			2	4				4
Total		39	27	3	6	3	38	0	0	1	39

TABLE VII

Site of Ligament Failure

	Number	Percentage
Tibial Avulsion	65	83
Femoral Avulsion	3	4
Midsubstance tear at sutures	6	8
Midsubstance tear not at sutures	4	5
Total	78	100

TABLE VIII

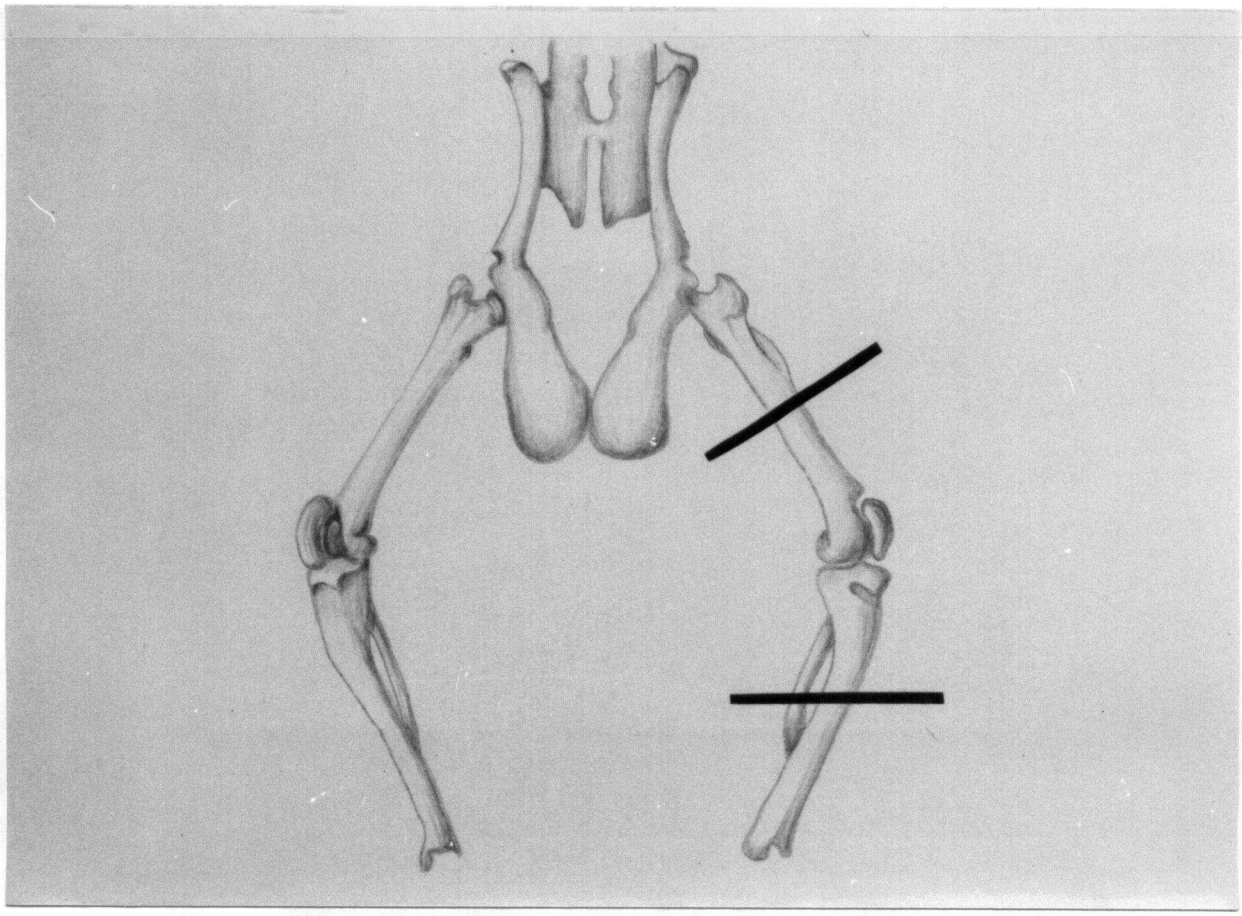


Figure 1: A drawing of the caudal one half of the appendicular skeleton of a rat, indicating the position of placement of the two Kirschner wires which penetrated both medial and lateral sides of the leg.

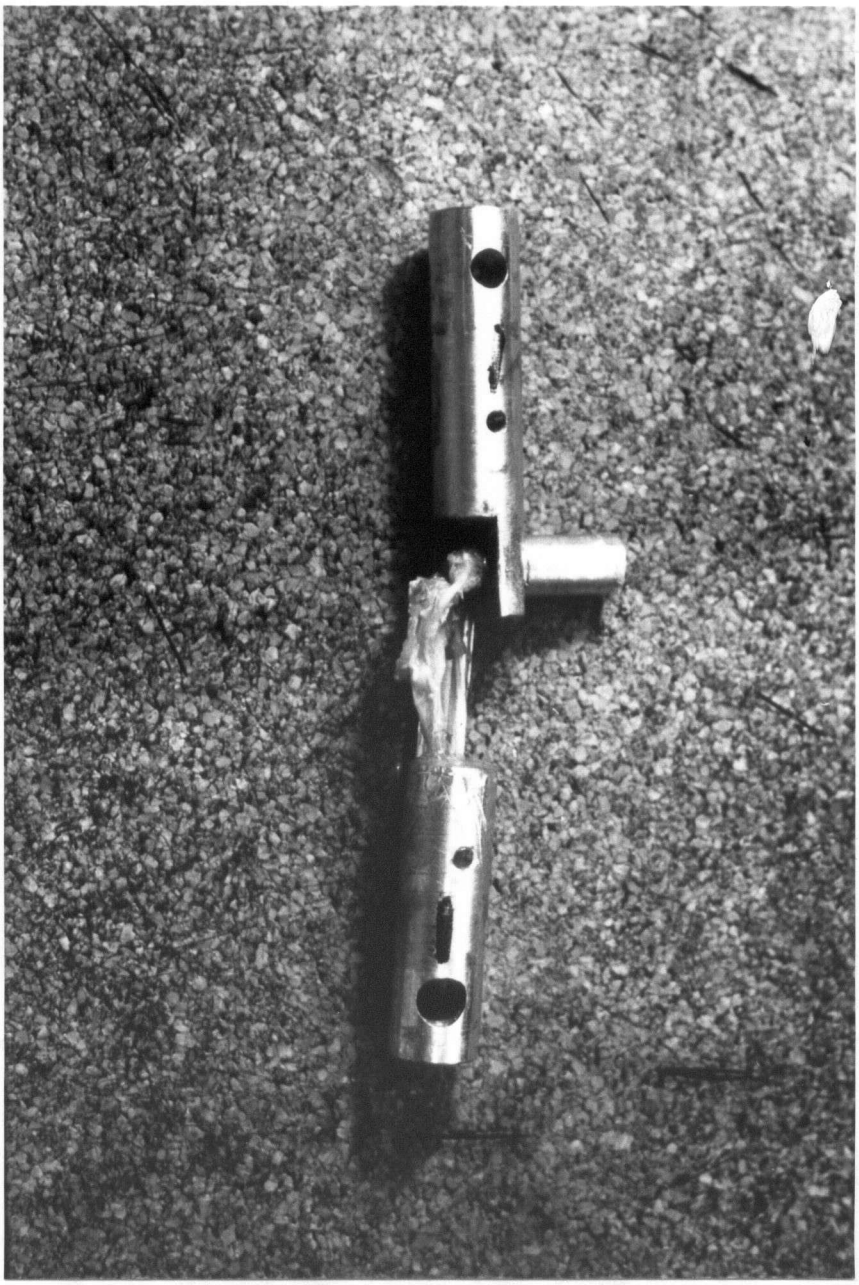


Figure 2: Special aluminum holders designed for testing of ligament-bone preparation in the Instron material testing machine. Femur-medial collateral ligament-tibia preparation has been set in epoxy in these holders. Note the removal bar bridging the two portions of the holder to stabilize the preparation prior to test.

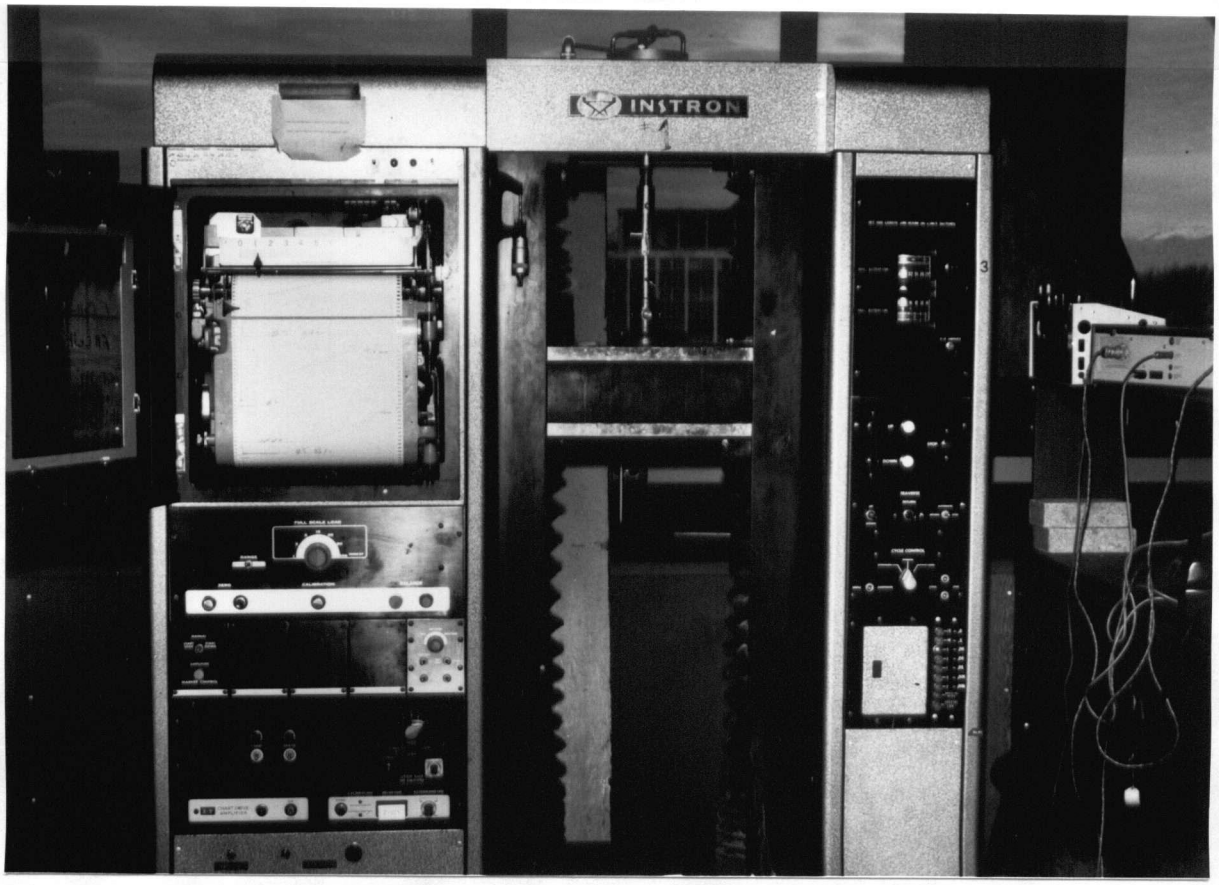
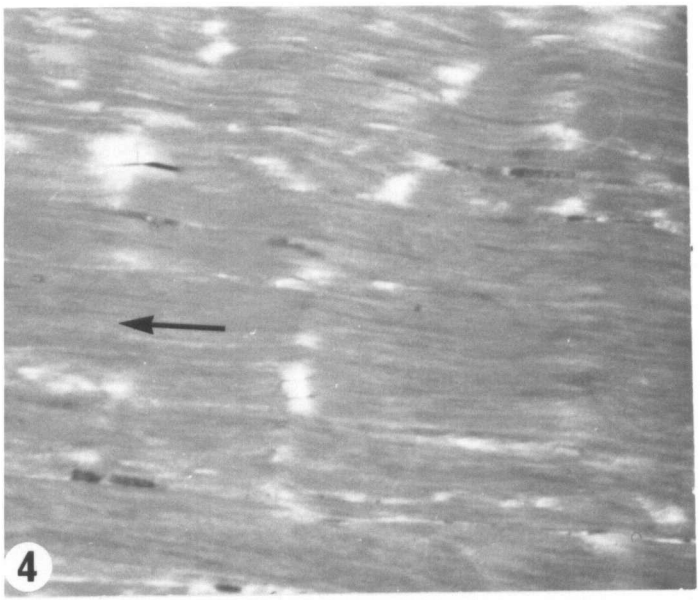


Figure 3: Instron materials testing machine.

Figure 4: Light micrograph of a control ligament. Arrow indicates direction of longitudinal ligament axis. Collagen fibers are densely packed and parallel to ligament axis. X 720.

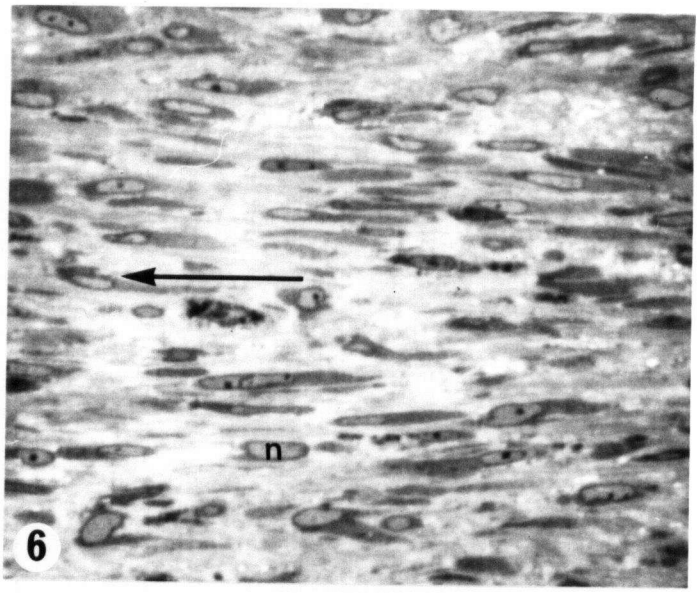
Figure 5: Light micrograph of a control ligament. Fibroblast nuclei (n) are arranged parallel to collagen fibers and are few in number. X 180.

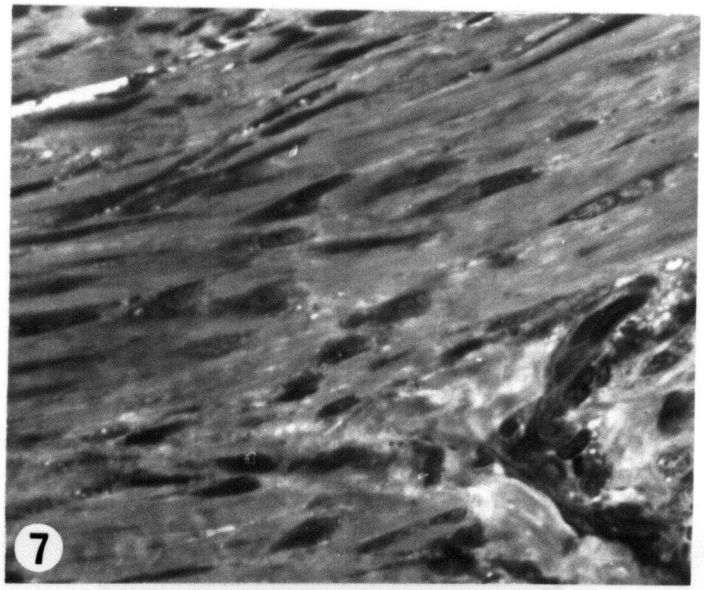


Control

Figure 6: Light micrograph of a repairing area of a ligament in Group A at 2 weeks post-operative. Arrow indicates ligament axis. There is a marked hypercellularity of parallel fibroblast nuclei (n). Collagen fiber formation is present. X 720.

Figure 7: Light micrograph of a repairing area of a ligament in Group A at 2 weeks post-operative. There is a marked hypercellularity of mainly parallel fibroblasts. Collagen fiber formation is present which has some attempt at parallel organization. X 720.

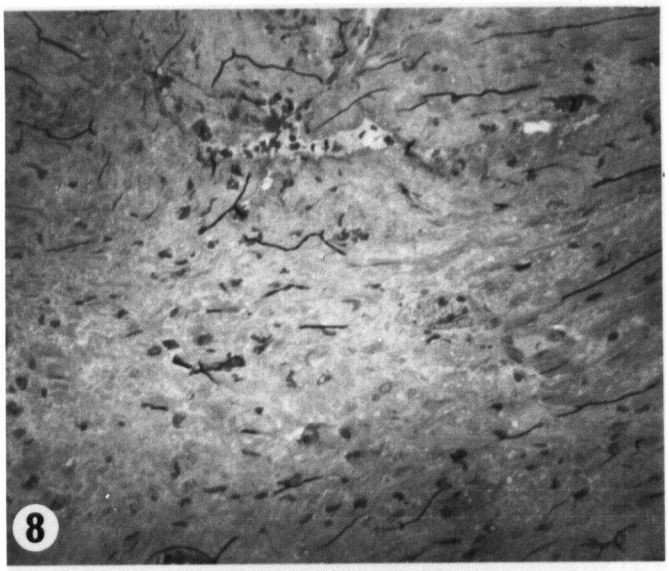


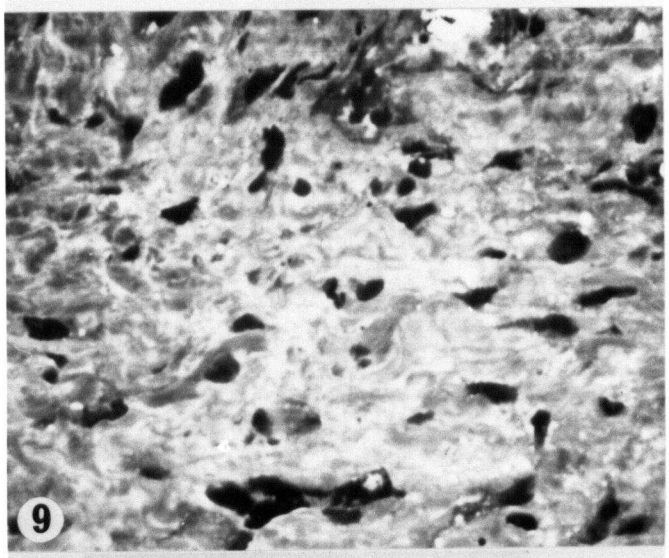


Group A 2wks.

Figure 8: Low power light micrograph of a repairing area of a ligament in Group B at 2 weeks post-operative. There is a hypercellularity of fibroblasts with no organization. Early collagen fiber formation is present with no organization. X 285.

Figure 9: Higher power light micrograph of the same area in Figure 8. Fibroblasts are large with dark staining nuclei and have no organization. Collagen is present, but in small amounts and without any organization. X 720.

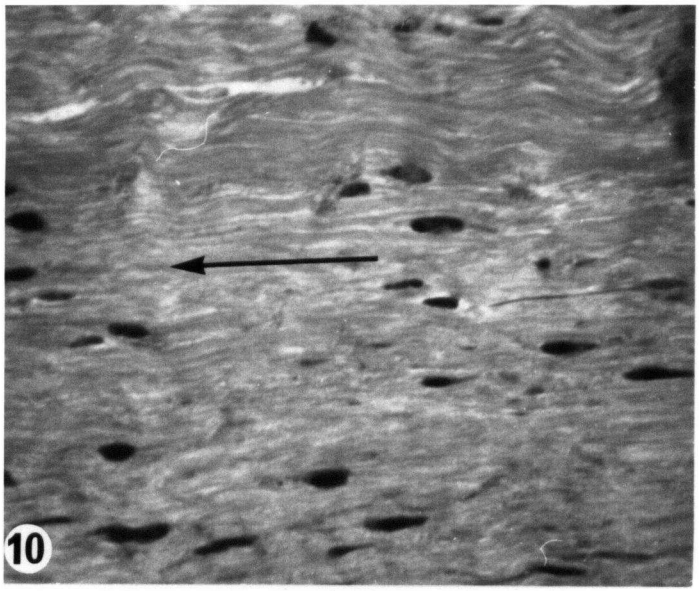


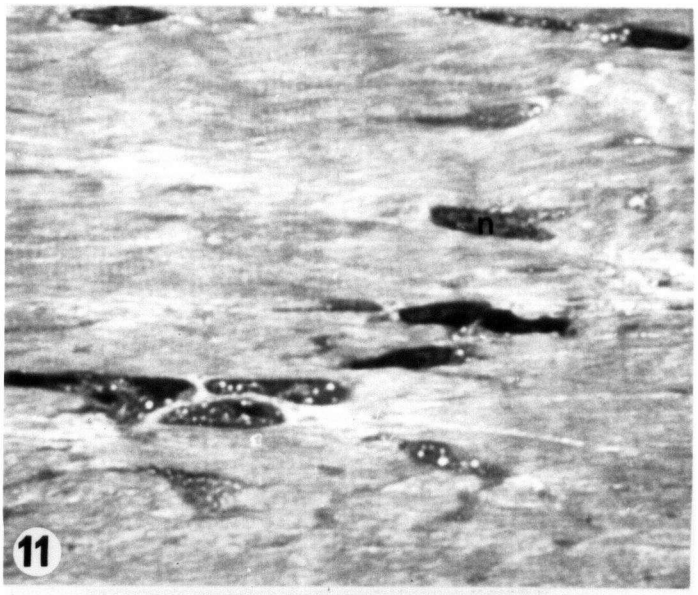


Group B 2wks.

Figure 10: Light micrograph of the repairing area of a ligament in Group A at 6 weeks post-operative. The number of fibroblasts has decreased and they are parallel to the ligament axis as indicated by the arrow. The collagen is densely packed and also parallel to the ligament axis. X 720.

Figure 11: High power light micrograph of the repairing area of a ligament in Group A at 6 weeks post-operative. Fibroblast nuclei (n) are seen in smaller numbers and parallel to the ligament axis. Collagen is dense and regular. X 1,800.

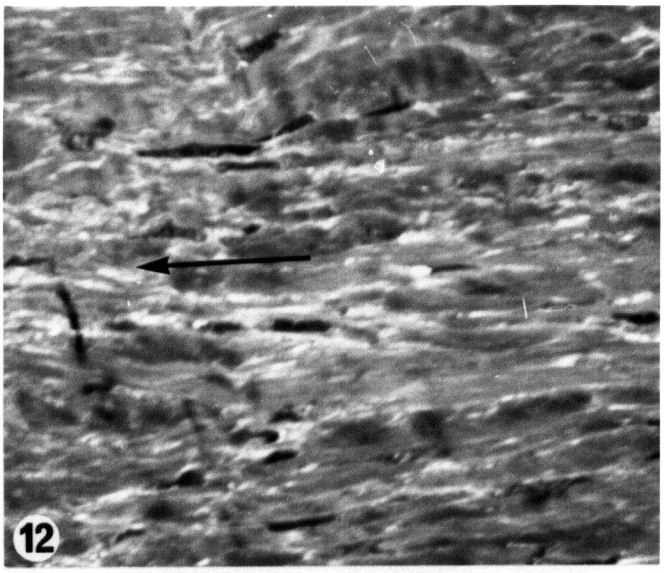


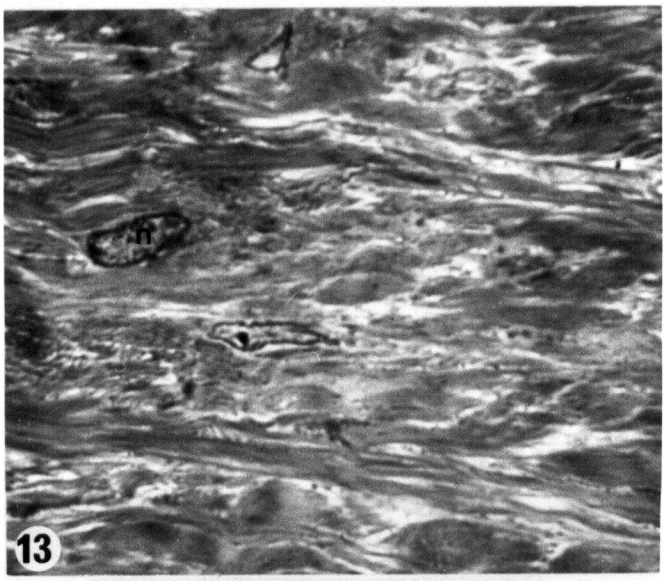


Group A 6wks.

Figure 12: Light micrograph of a repairing area of a ligament in Group B at 6 weeks post-operative. The fibroblasts have decreased in numbers and are now parallel to the ligament axis as indicated by the arrow. The collagen has increased in amount and is now mainly parallel to the ligament axis. X 720.

Figure 13: Light micrograph of a repairing area of a ligament in Group B at 6 weeks post-operative. The fibroblast nuclei (n) have decreased in numbers and are parallel to the ligament axis. The collagen fibers have some organization although not as much as in Group A. The collagen is not as densely packed as in Group A. X 1,800.

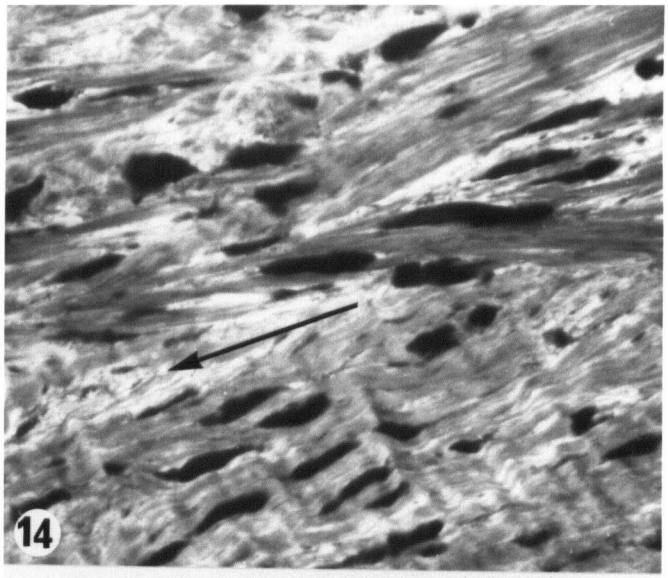


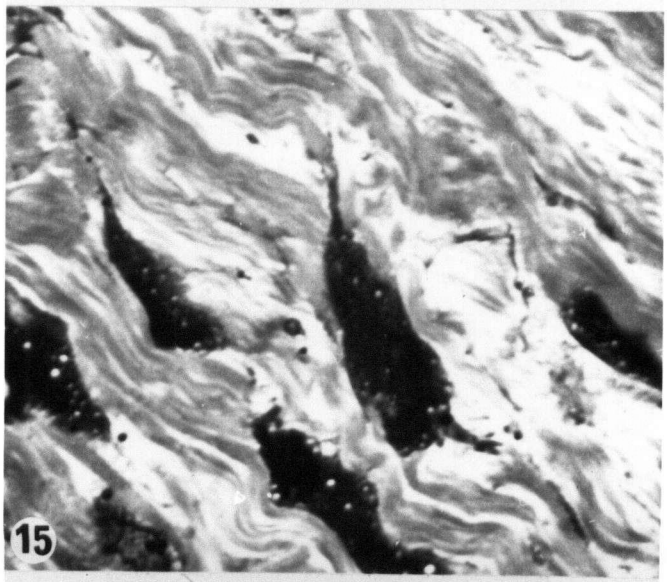


Group B 6wks.

Figure 14: Light micrograph of a repairing area of a ligament in Group C at 6 weeks post-operative. Fibroblast nuclei are large, darkly staining and have some organization in relation to the ligament axis as indicated by the arrow. Collagen fibers are loosely packed and also have some organization although fiber branching from the ligament axis is seen. X 720.

Figure 15: Light micrograph of a repairing area of a ligament in Group C at 6 weeks post-operative. Numerous large fibroblasts are seen with large darkly staining nuclei and abundant intracytoplasmic vesicles. Collagen fibers are loosely packed. X 1,800.

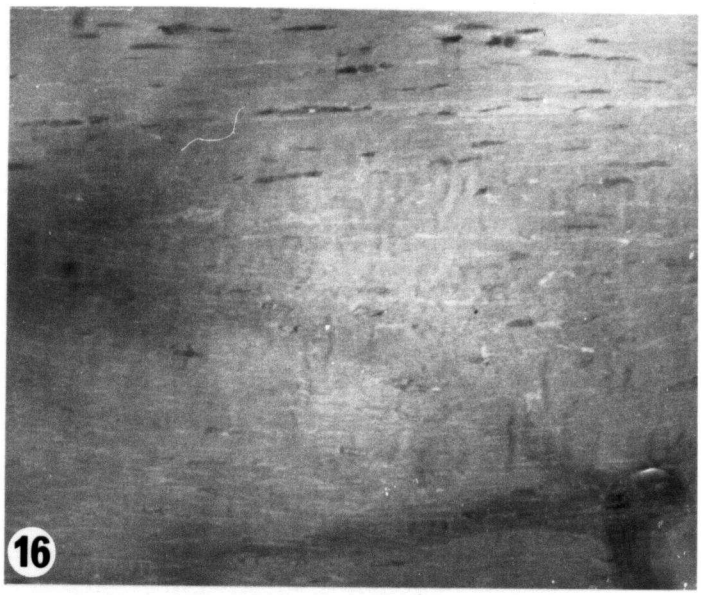


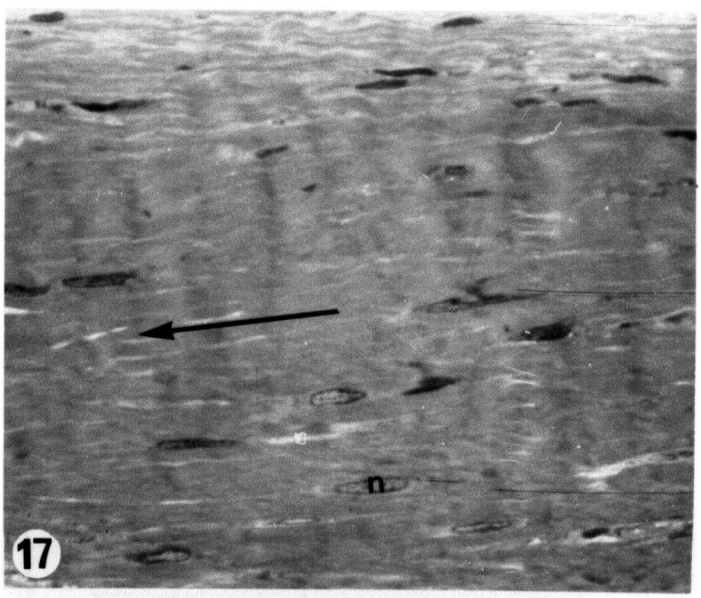


Group C 6wks.

Figure 16: Light micrograph of a repairing area of a ligament in Group A at 10 weeks post-operative. Fibroblast nuclei have decreased in number and are all parallel. Collagen fibers are densely packed and regular. X 285.

Figure 17: Light micrograph of a repairing area of a ligament in Group A at 10 weeks post-operative. Fibroblast nuclei (n) are small in number and parallel to the ligament axis as indicated by the arrow. Collagen fibers are densely packed, regular and well organized. X 720.

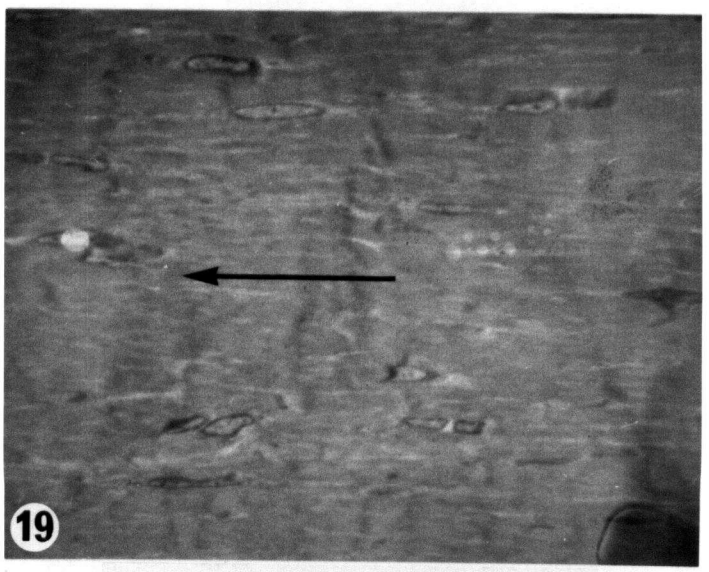




Group A 10wks.

Figure 18: Light micrograph of a repairing area of a ligament in Group B at 10 weeks post-operative. Fibroblast nuclei are few in number and parallel. Collagen fibers are densely packed and well organized. X 720.

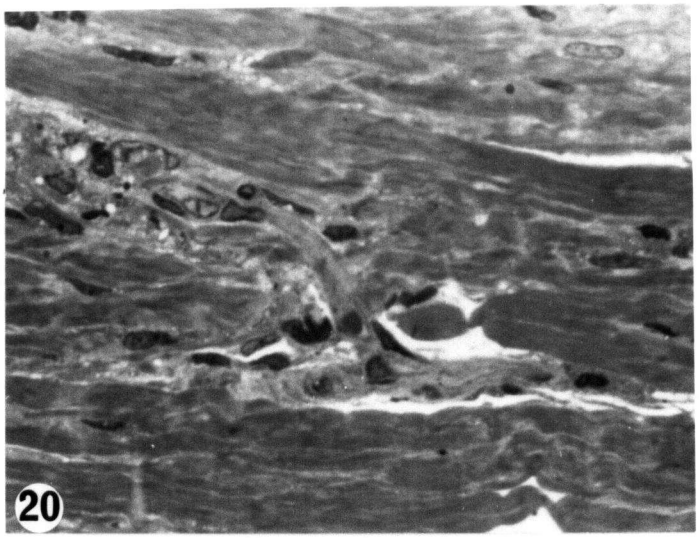
Figure 19: Light micrograph of a repairing area of a ligament in Group B at 10 weeks post-operative. Fibroblast nuclei are parallel to the ligament axis as indicated by the arrow. The collagen fibers are also parallel to this axis and are densely packed and well organized. X 720.

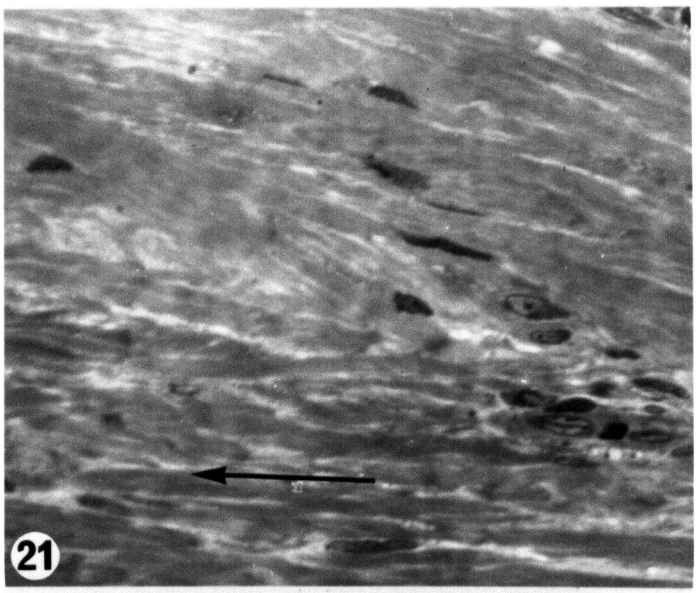


Group B 10wks.

Figure 20: Light micrograph of a repairing area of a ligament in Group C at 10 weeks post-operative. Fibroblast nuclei have decreased in numbers and are better organized. Collagen fibers are packed densely and are better organized than at 6 weeks. X 720.

Figure 21: Light micrograph of a repairing area of a ligament in Group C at 10 weeks post-operative. Fibroblast nuclei have decreased in numbers. Collagen fibers are packed denser than at 6 weeks but still deviate from the ligament axis as indicated by the arrow. X 720.

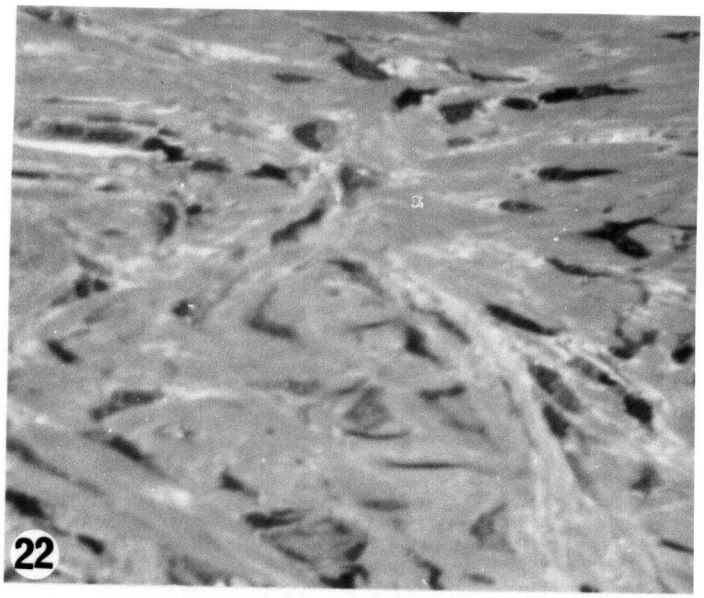


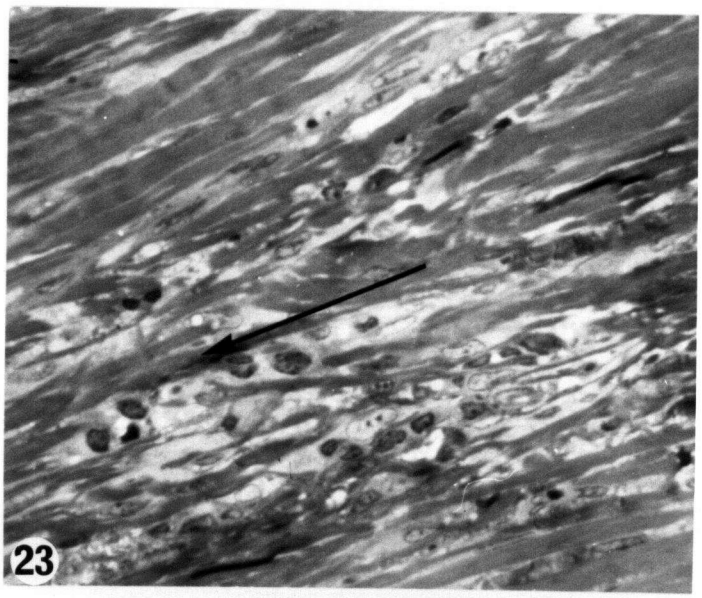


Group C 10wks.

Figure 22: Light micrograph of a repairing area of a ligament in Group D at 10 weeks post-operative. Fibroblast nuclei are numerous, large and poorly organized. X 720.

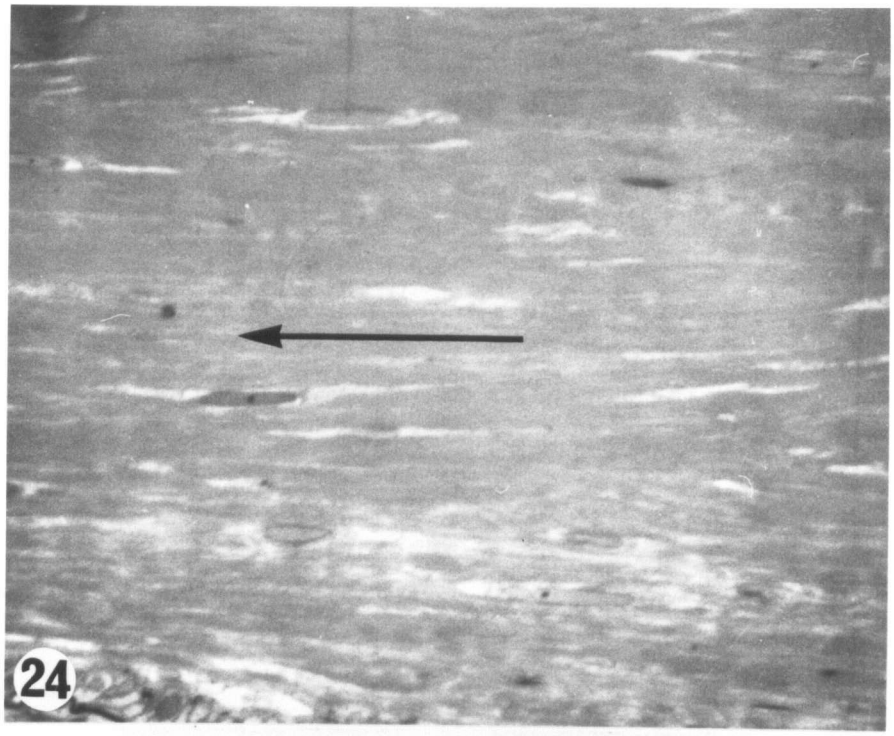
Figure 23: Light micrograph of a repairing area of a ligament in Group D at 10 weeks post-operative. Fibroblast nuclei here show some organization but a large variability in size. Collagen is packed loose in this area but with some longitudinal organization to the ligament axis as indicated by the arrow. Collagen branching from this axis is seen. X 720.





Group D 10wks.

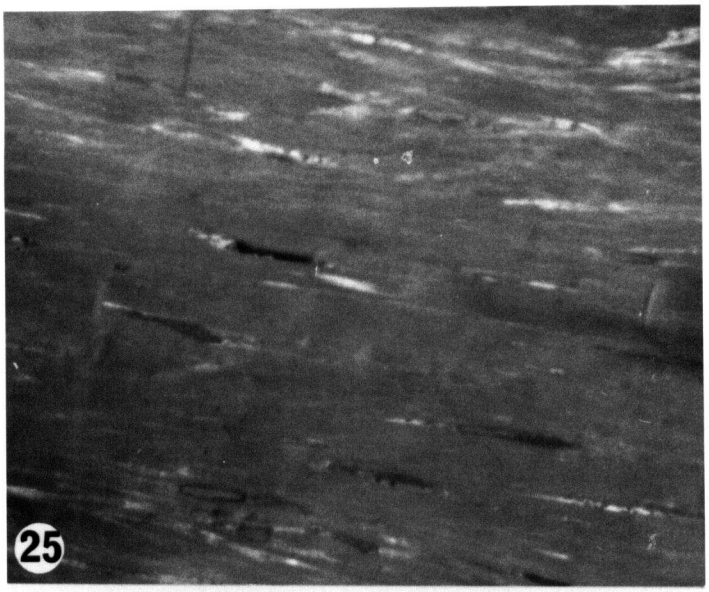
Figure 24: Light micrograph of a repairing area of a ligament in Group A at 20 weeks post-operative. Fibroblast nuclei are few in number and parallel to the longitudinal ligament axis as indicated by the arrow. The collagen fibers are densely packed, regular and parallel to the ligament axis. The appearance is the same as the controls. X 720.

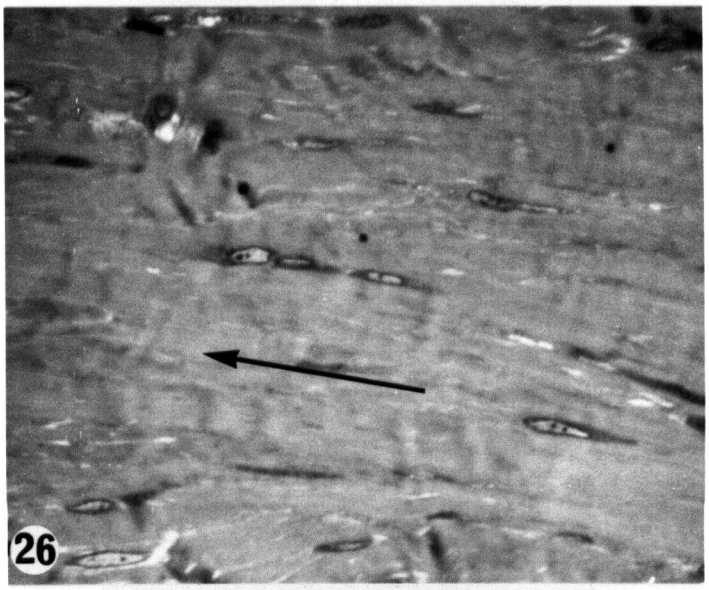


Group A 20wks.

Figure 25: A light micrograph of the repairing area of a ligament in Group B at 20 weeks post-operative. Fibroblast nuclei are more numerous than in Group A and are parallel to the ligament axis. Collagen fibers are densely packed and regular. X 720.

Figure 26: Light micrograph of a repairing area of a ligament in Group B at 20 weeks post-operative. Fibroblast nuclei are parallel to the ligament axis as indicated by the arrow and are more numerous than in Group A. Collagen fibers are also parallel to the axis and well organized. X 720.

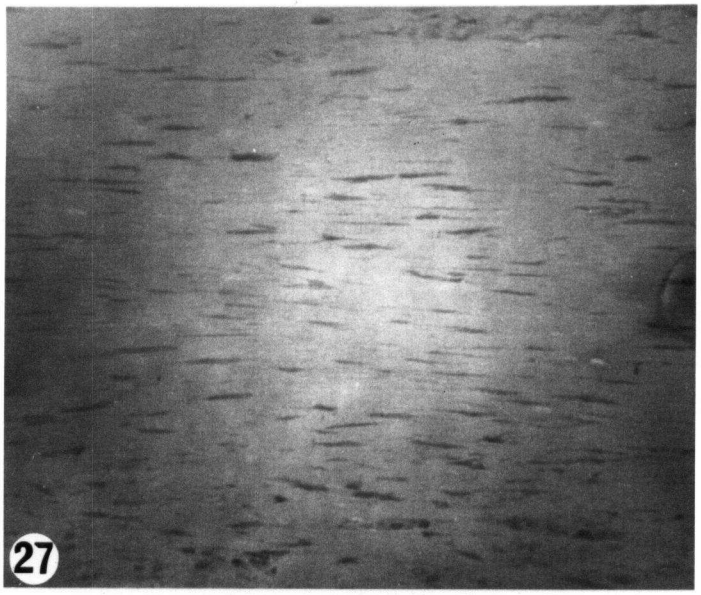




Group B 20wks.

Figure 27: Light micrograph of a repairing area of a ligament in Group C at 20 weeks post-operative. Fibroblast nuclei remain numerous and are parallel and longitudinal. The collagen fibers are densely packed and regular. X 285.

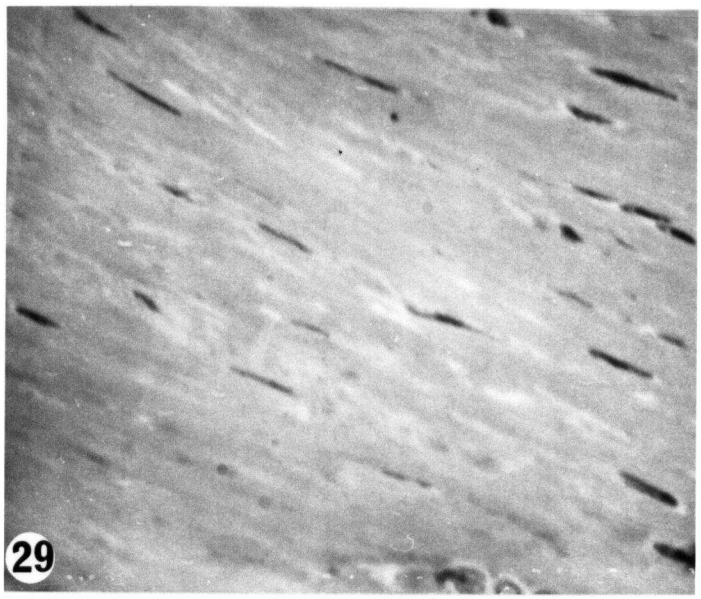
Figure 28: Light micrograph of a repairing area of a ligament in Group C at 20 weeks post-operative. Fibroblast nuclei are numerous and parallel to the ligament axis as indicated by the arrow. The collagen fibers are densely packed, regular and parallel to the ligament axis. X 720.

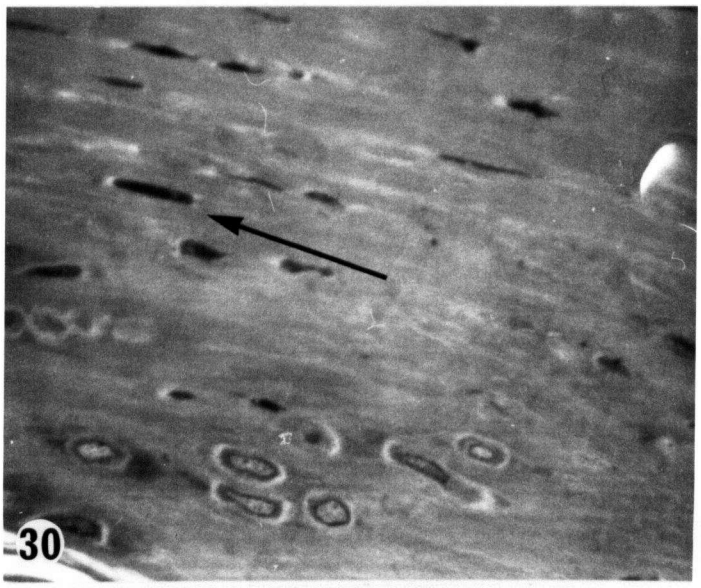


Group C 20wks.

Figure 29: Light micrograph of a repairing area of a ligament in Group D at 20 weeks post-operative. Fibroblast nuclei have decreased in number, but still are more numerous than in controls, and are now mainly parallel to the ligament axis. The collagen fibers are densely packed and regular. X 720.

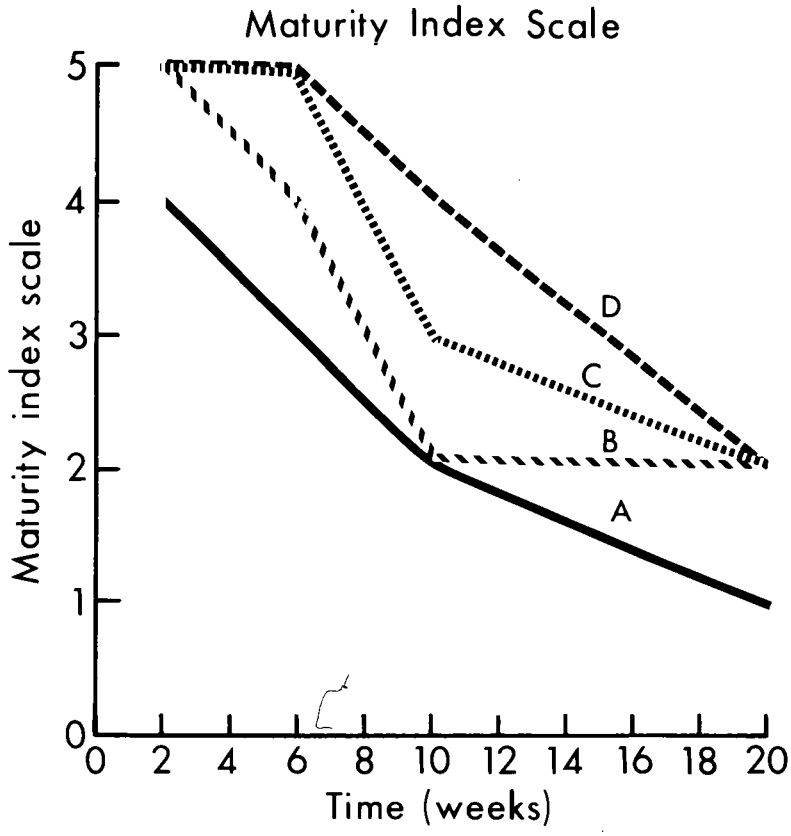
Figure 30: Light micrograph of a repairing area of a ligament in Group D at 20 weeks post-operative. Fibroblast nuclei are numerous and parallel to the ligament axis as indicated by the arrow. The collagen fibers are densely packed and mainly parallel to this axis although some branching is seen. X 720.





Group D 20wks.

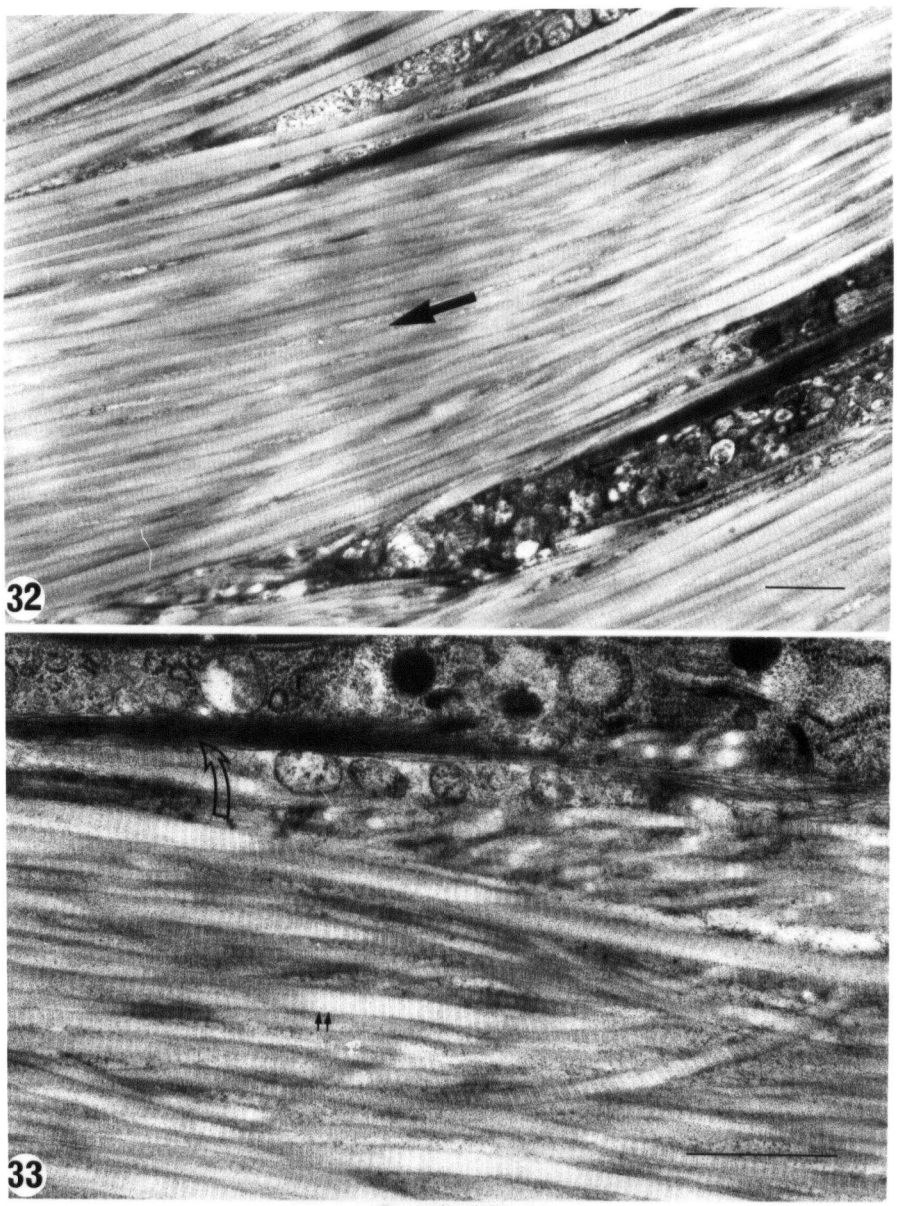
Figure 31: A graph of the results of the Maturity Index Score as applied to the Maturity Index Scale versus time (in weeks) post-operative. Group A has the most rapid maturation and achieves full maturity at 20 weeks post-operative. All of the other groups show a decreased rate of maturation which is more pronounced while their limbs are immobilized. At 20 weeks post-operative Groups B, C and D are all in the late remodelling phase of healing.


Maturity Index Scale
Maturity index scale
5
4
3
2
1
0
0 2 4 6 8 10 12 14 16 18 20
Time (weeks)
D
C
B
A

31

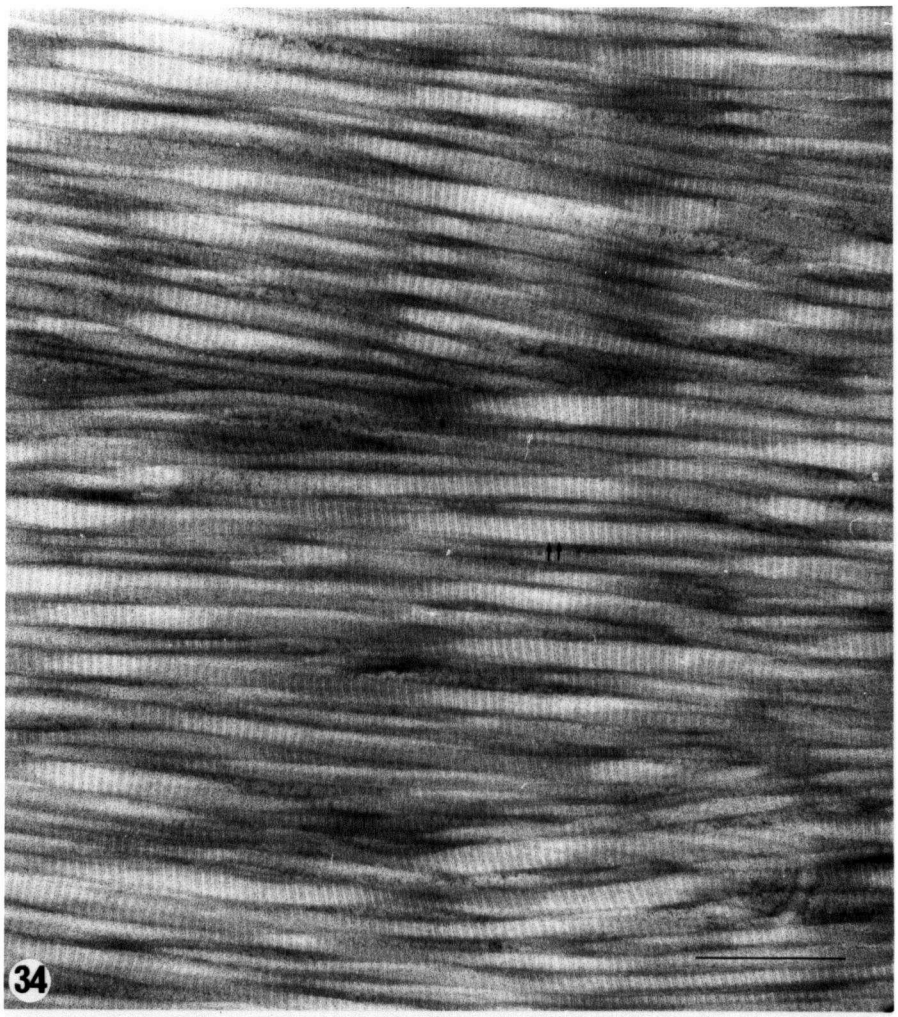
Figure 32: Electron micrograph of a control ligament. A one micron bar marker is seen in lower right corner. The fibroblasts are small in size and not very active metabolically. The collagen fibers are densely packed, regular and parallel to the longitudinal ligament axis as indicated by the solid arrow. X 14,300.

Figure 33: Electron micrograph of a control ligament with a one micron bar marker in the lower right corner. Rough endoplasmic reticulum (r) is seen intracellularly in the fibroblast and tropocollagen fibril formation is seen at the cell periphery (open arrow). The regular 640A banding of the collagen fibers is seen (two small arrows). X 28,000.



Control

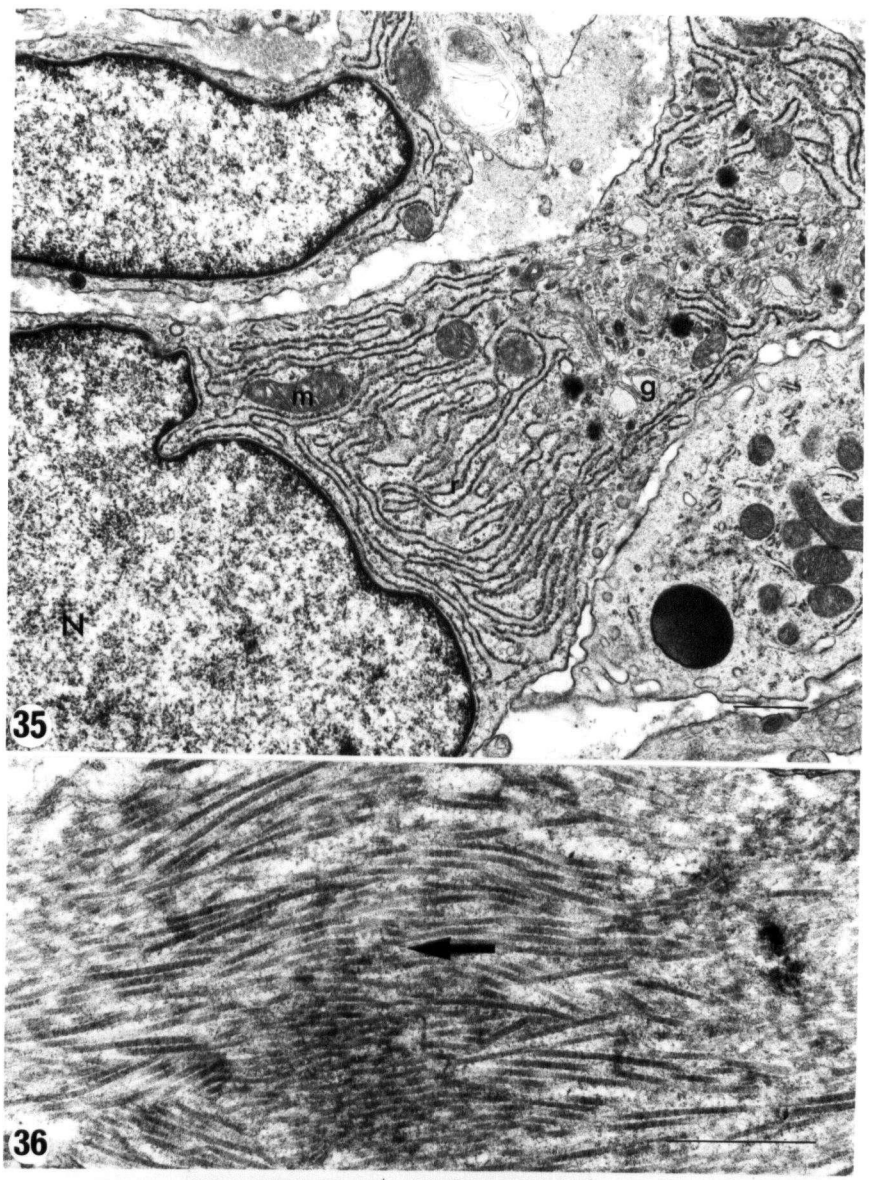
Figure 34: Electron micrograph of a control ligament. A one micron bar marker is in the lower right corner. The regularity and dense packing of the collagen fibers is seen. The 640Å banding is indicated by the two small arrows. X 28,000.



Control

Figure 35: Electron micrograph of a repairing area of a ligament in Group A at 2 weeks post-operative. A one micron bar marker is in the lower right corner. The fibroblast nucleus (N) is large with clumps of chromatin seen. The cell is very large and metabolically active with abundant mitochondria (m), rough endoplasmic reticulum (r) and golgi complexes (g). X 14,300.

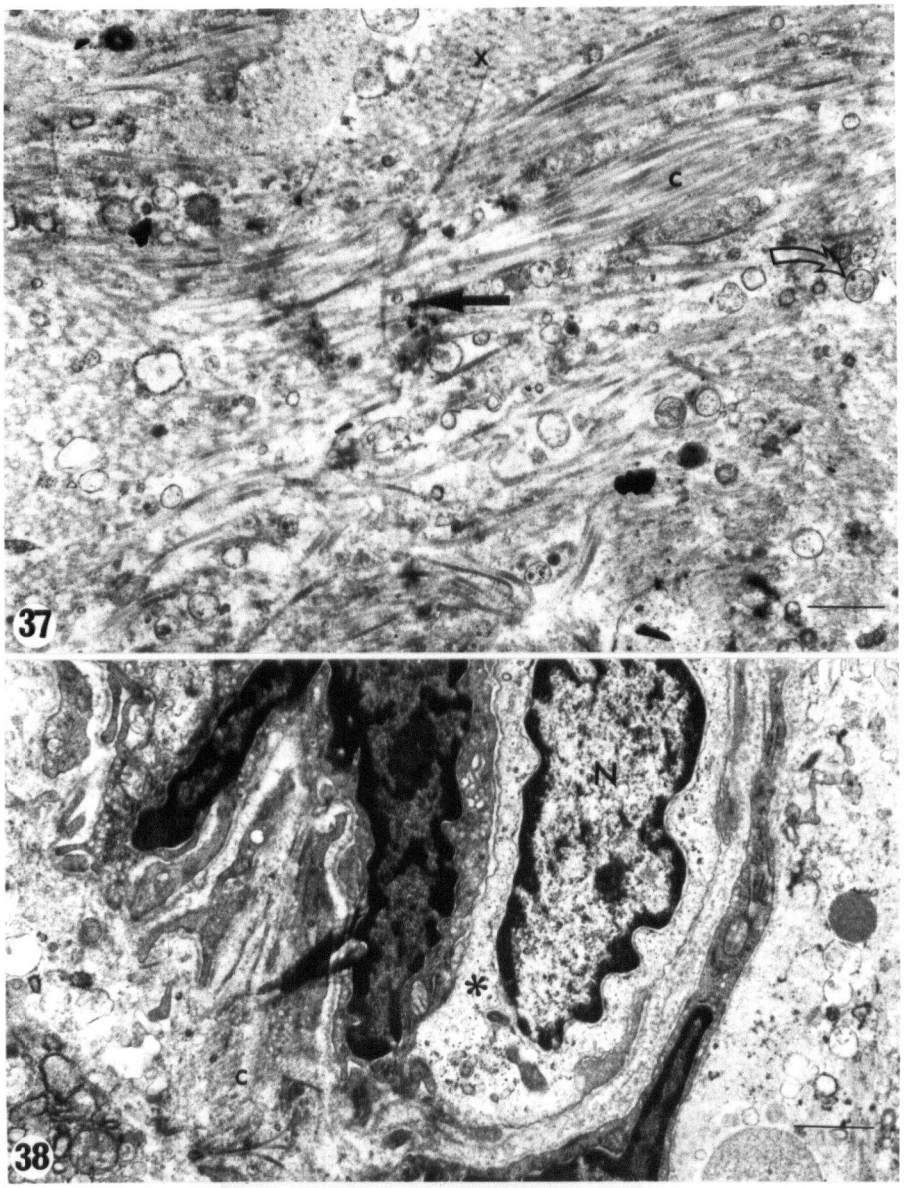
Figure 36: Electron micrograph of a repairing area of a ligament in Group A at 2 weeks post-operative. A one micron bar marker is seen in the lower right corner. The collagen fibers are seen to be forming in an organized fashion parallel to the ligament axis as indicated by the large solid arrow. The collagen is loosely packed and small in diameter. X 28,000.



Group A 2wks.

Figure 37: Electron micrograph of a repairing area of a ligament in Group B at 2 weeks post-operative. The ligament axis is indicated by the large solid arrow. The collagen fibers (c) are seen to have no organization and can even be seen in cross-section (X). Large amounts of extracellular debris are seen (open arrow). A one micron bar marker is in the lower right corner. X 14,3000.

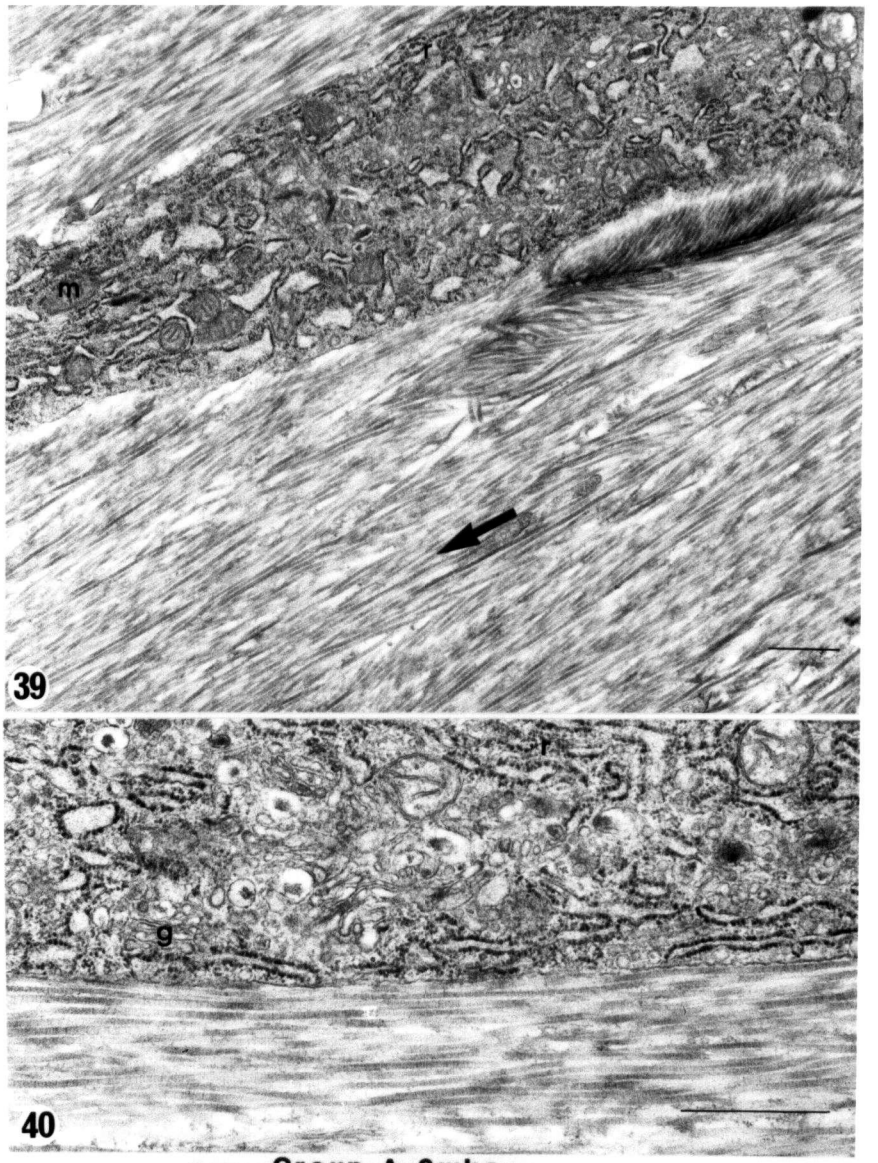
Figure 38: Electron micrograph of a repairing area of a ligament in Group B at 2 weeks post-operative. A smaller fibroblast nucleus (N) is seen. The cell is not very active metabolically as indicated by the lack of intracellular organelles (asterix). Some collagen fibers (c) are seen. A one micron bar marker is in the lower right corner. X 14,300.



Group B 2wks.

Figure 39: Electron micrograph of a repairing area of a ligament in Group A at 6 weeks post-operative. The fibroblasts are still large and metabolically active with abundant mitochondria (m) and rough endoplasmic reticulum (r). The collagen fibers are packed more densely and are parallel to the ligament axis (large solid arrow). A one micron bar marker is in the lower right corner. X 14,300.

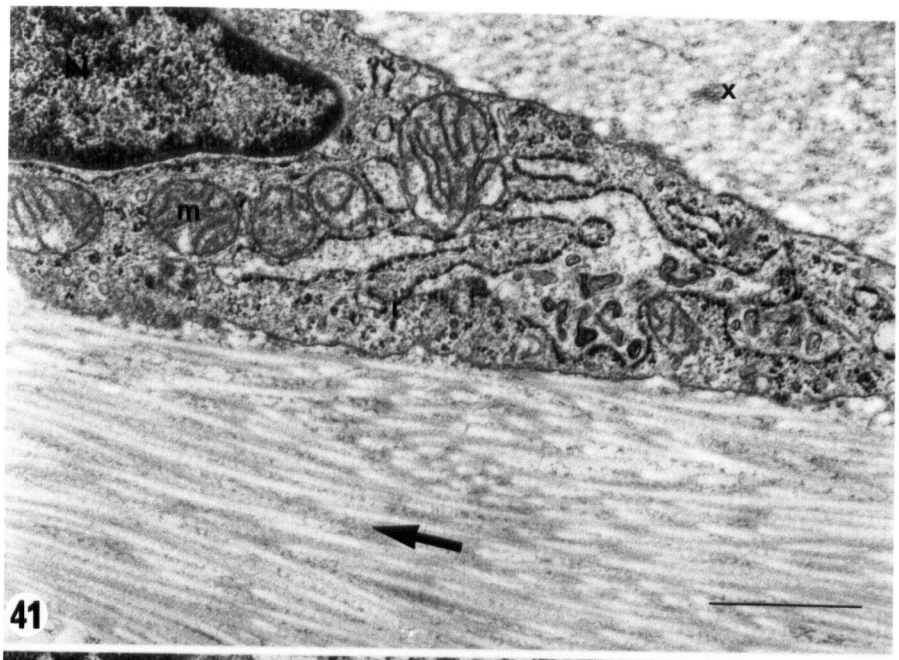
Figure 40: Higher power electron micrograph of the same area seen in Figure 39. Numerous golgi complexes (g) are seen. The collagen fibers are very regular and more densely packed than at 2 weeks. A one micron bar marker is in the lower right corner. X 28,000.

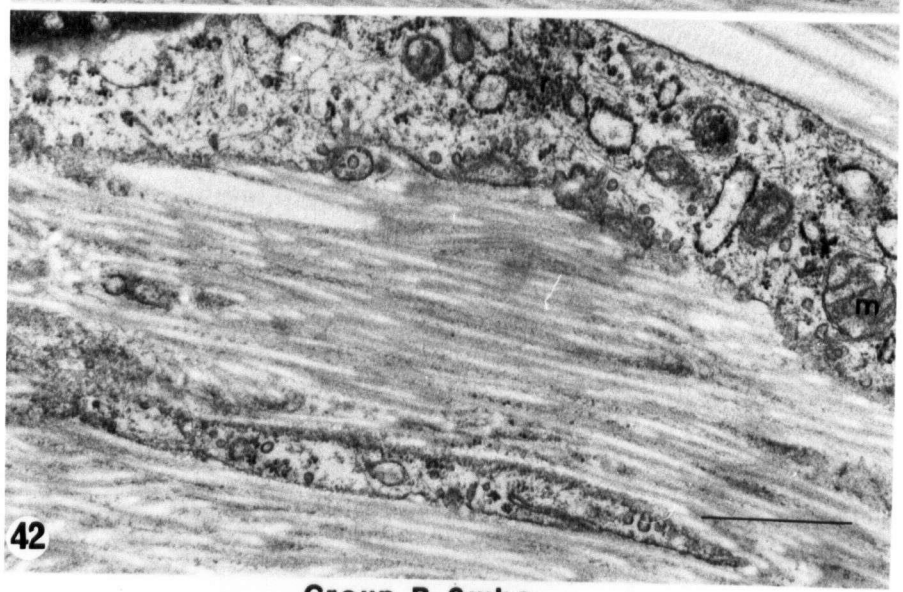


Group A 6wks.

Figure 41: Electron micrograph of a repairing area of a ligament in Group B at 6 weeks post-operative. The fibroblast has increased in size and is more active metabolically than at 2 weeks with abundant mitochondria (m) and rough endoplasmic reticulum (r). Collagen fibers are seen more densely packed and with some organization when compared to the ligament axis (large arrow) although fibers in cross-section (X) are seen in the same area. One micron bar marker in lower right corner. X 28,000.

Figure 42: Electron micrograph of a repairing area of a ligament in Group B at 6 weeks post-operative. A large fibroblast with abundant mitochondria (m) and rough endoplasmic reticulum (r) is seen. A one micron bar marker is in the lower right corner. X 28,000.

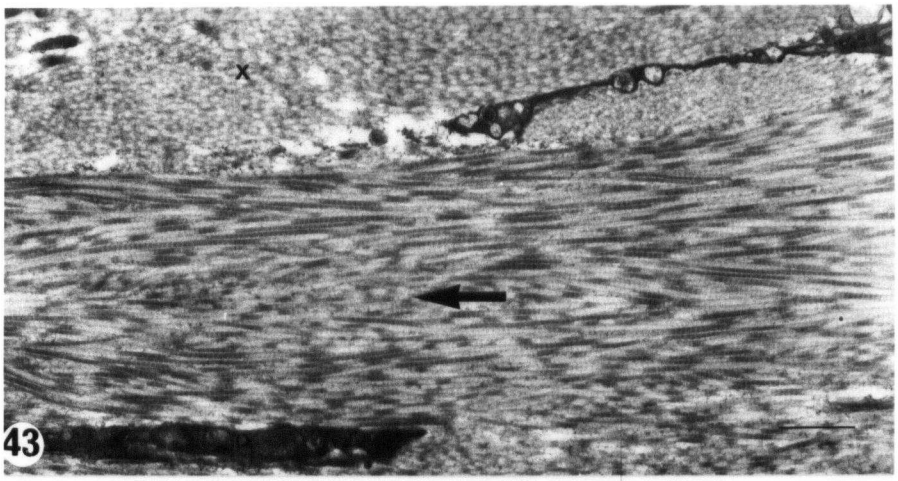


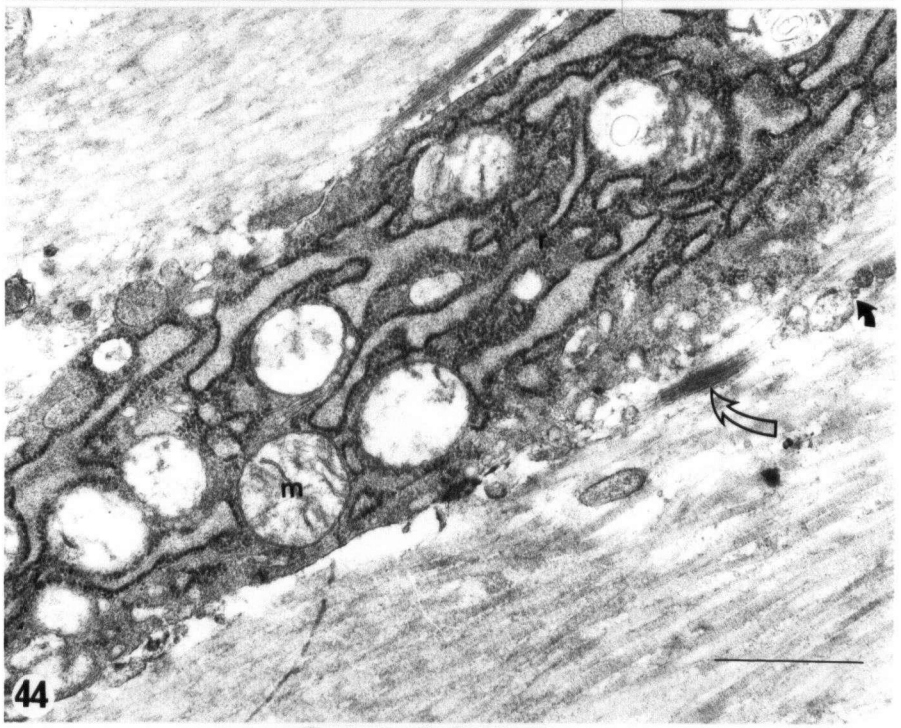


Group B 6wks.

Figure 43: Electron micrograph of a repairing area of a ligament in Group C at 6 weeks post-operative. The ligament axis is indicated by the solid arrow. The collagen has some organization although fibers in cross-section (X) are seen beside longitudinal ones. A one micron bar marker is seen in the lower right corner. X 14,300.

Figure 44: Electron micrograph of a repairing area of a ligament in Group C at 6 weeks post-operative. A portion of a large fibroblast is seen which is very active metabolically. There is abundant mitochondria (m) and rough endoplasmic reticulum (r). Tropocollagen filaments (open arrow) are seen at the cell margin. Extracellular debris (small solid arrow) is present. A one micron bar marker is in the lower right corner. X 28,000.

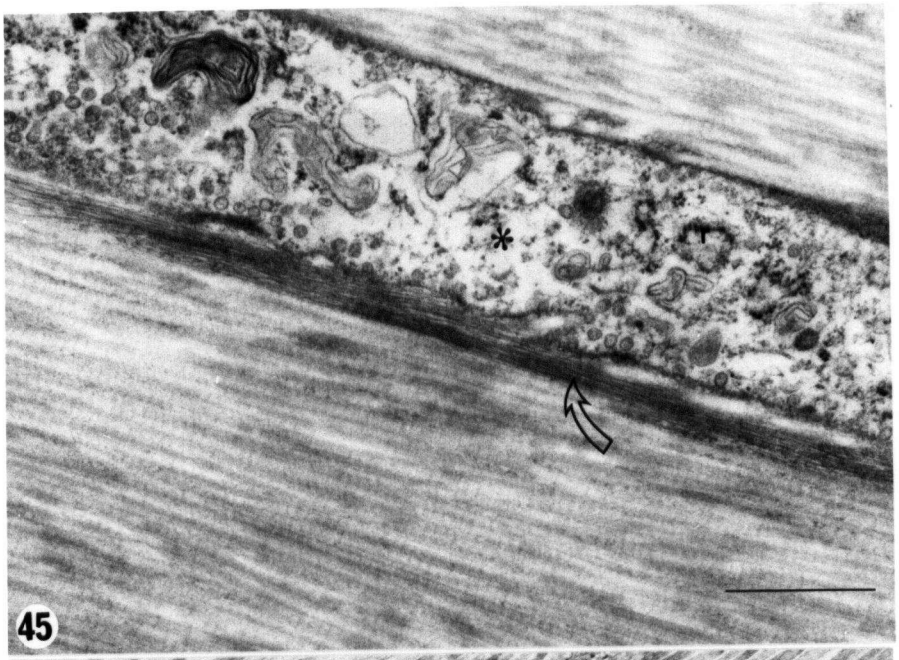


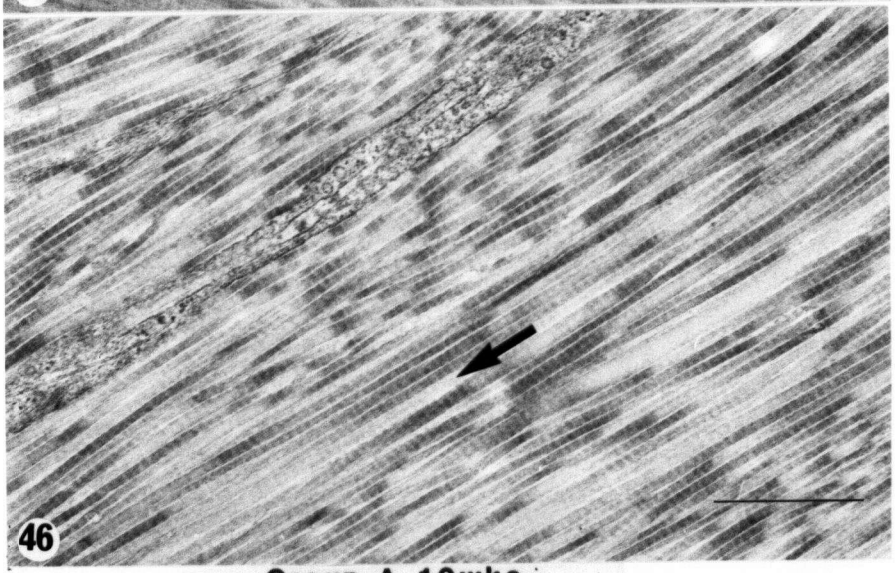


Group C 6wks.

Figure 45: Electron micrograph of a repairing area of a ligament in Group A at 10 weeks post-operative. The fibroblasts are less active metabolically as indicated by fewer intracellular organelles (asterix). Rough endoplasmic reticulum (r) is present and tropocollagen filaments are seen at the cell boundary (open arrow). A one micron bar marker is in the lower right corner. X 28,000.

Figure 46: Electron micrograph of a repairing area of a ligament in Group A at 10 weeks post-operative. The collagen fibers are densely packed, regular and longitudinally oriented parallel to the ligament axis (as indicated by the solid arrow). A one micron bar marker is in the lower right corner. X 28,000.

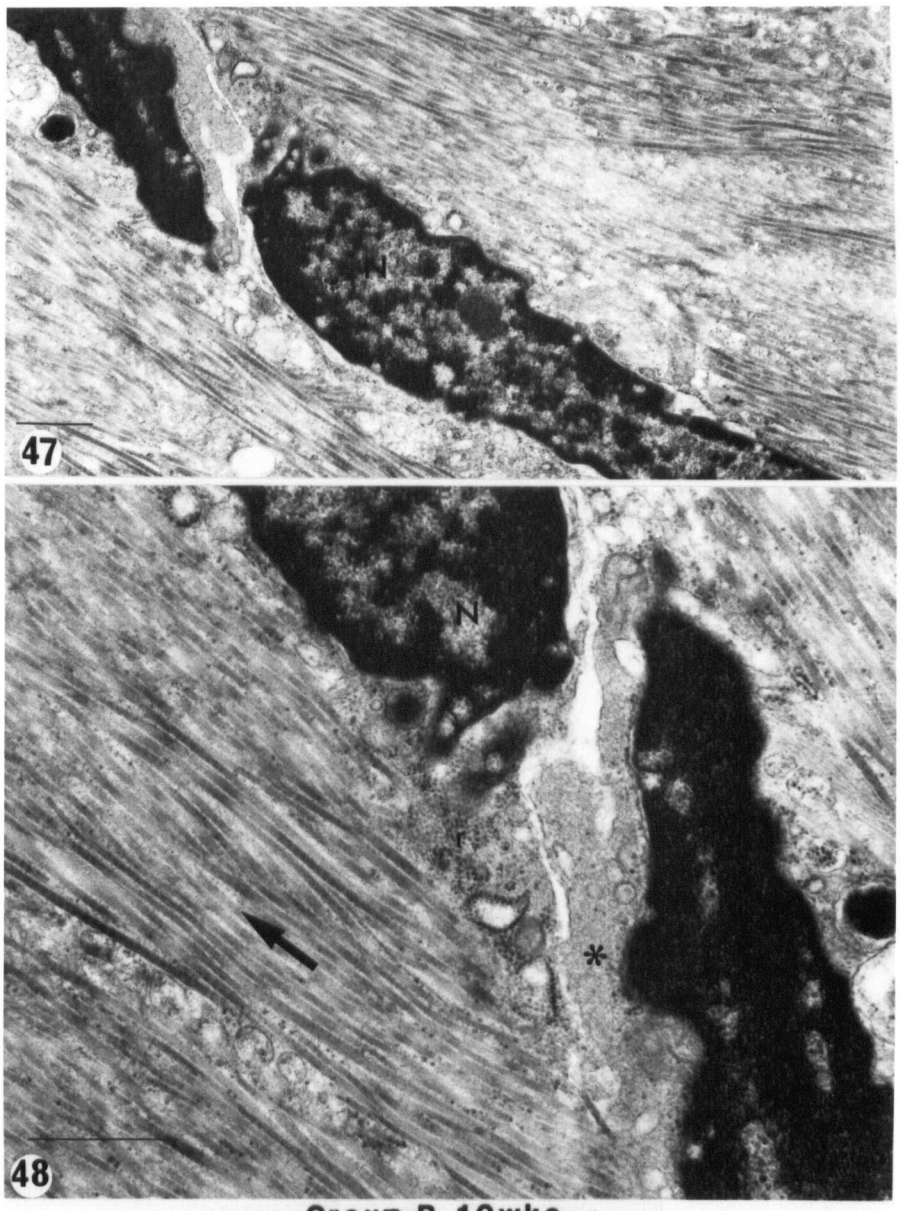




Group A 10wks.

Figure 47: Electron micrograph of a repairing area of a ligament in Group B at 10 weeks post-operative. A small fibroblast nucleus is seen (N). Collagen fibers are still branching and smaller in size than Group A. A one micron bar marker is in the lower left corner. X 14,300.

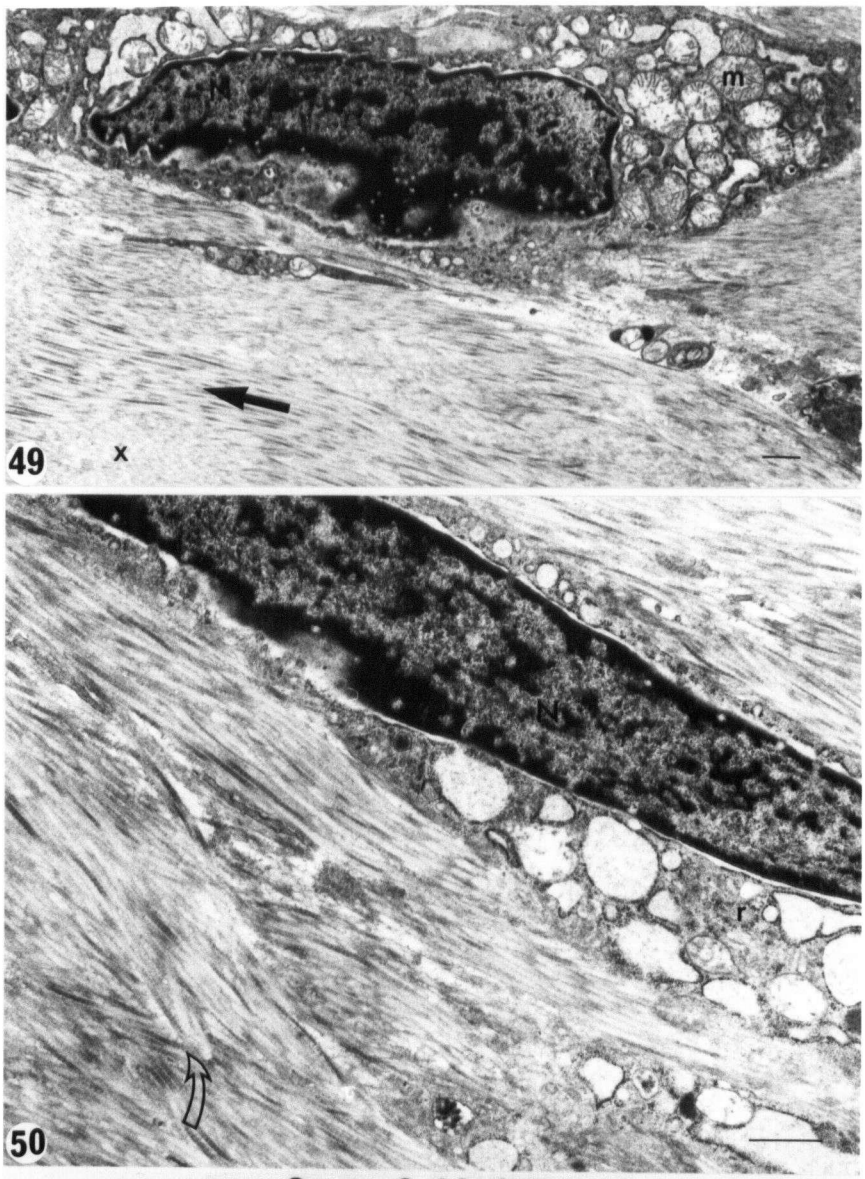
Figure 48: Electron micrograph of a repairing area of a ligament in Group B at 10 weeks post-operative. Two fibroblast nuclei are seen (n) and the cells are not very active metabolically as indicated by fewer intracellular organelles (asterix). Rough endoplasmic reticulum is seen (r). The collagen fibers have increased in organization although they still are not as densely packed or regular as Group A. The ligament axis is indicated by the large solid arrow. A one micron bar marker is in the lower left corner. X 28,000.



Group B 10wks.

Figure 49: An electron micrograph of a repairing area of a ligament in Group C at 10 weeks post-operative. A large fibroblast with a large nucleus (N) is seen. This fibroblast is metabolically very active with large numbers of mitochondria (m). The collagen fibers are not as well organized with longitudinal fibers and fibers in cross-section (X) seen. The large arrow indicates the ligament axis. A one micron bar marker is in the lower right corner. X 7,500.

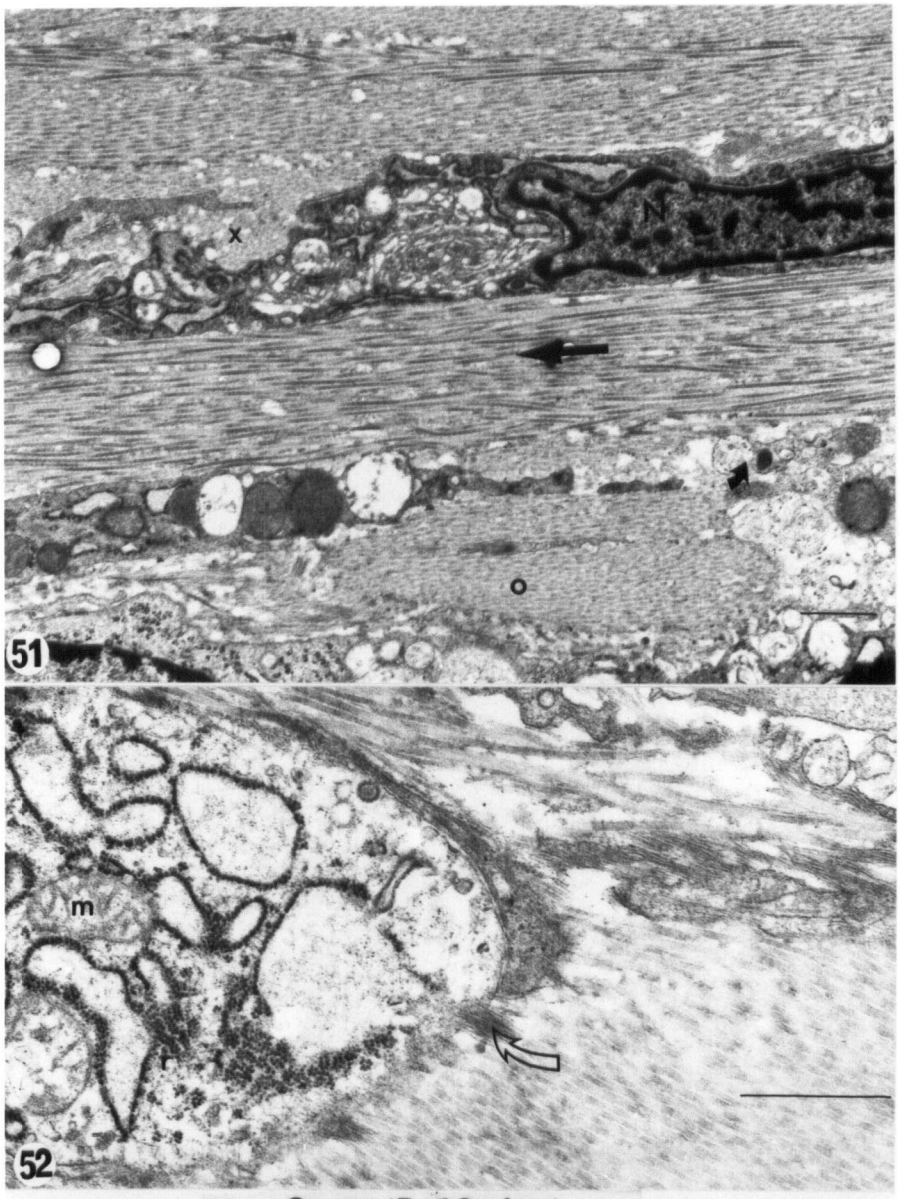
Figure 50: An electron micrograph of a repairing area of a ligament in Group C at 10 weeks post-operative. A large metabolically active fibroblast with rough endoplasmic reticulum (r) is seen. As seen in Figure 49 the collagen is not as well organized as Groups A or B and displays extensive branching (open arrow). A one micron bar marker is in the lower right corner. X 14,300.



Group C 10wks.

Figure 51: Electron micrograph of a repairing area of a ligament in Group D at 10 weeks post-operative. A small fibroblast nucleus (N) is seen and the fibroblast is not very active metabolically. Some of the collagen fibers are longitudinally oriented (large solid arrow) while others are seen in cross-section (X) and oblique (o). Extracellular debris is still present (small solid arrow). A one micron bar marker is seen in the lower right corner. X 14,300.

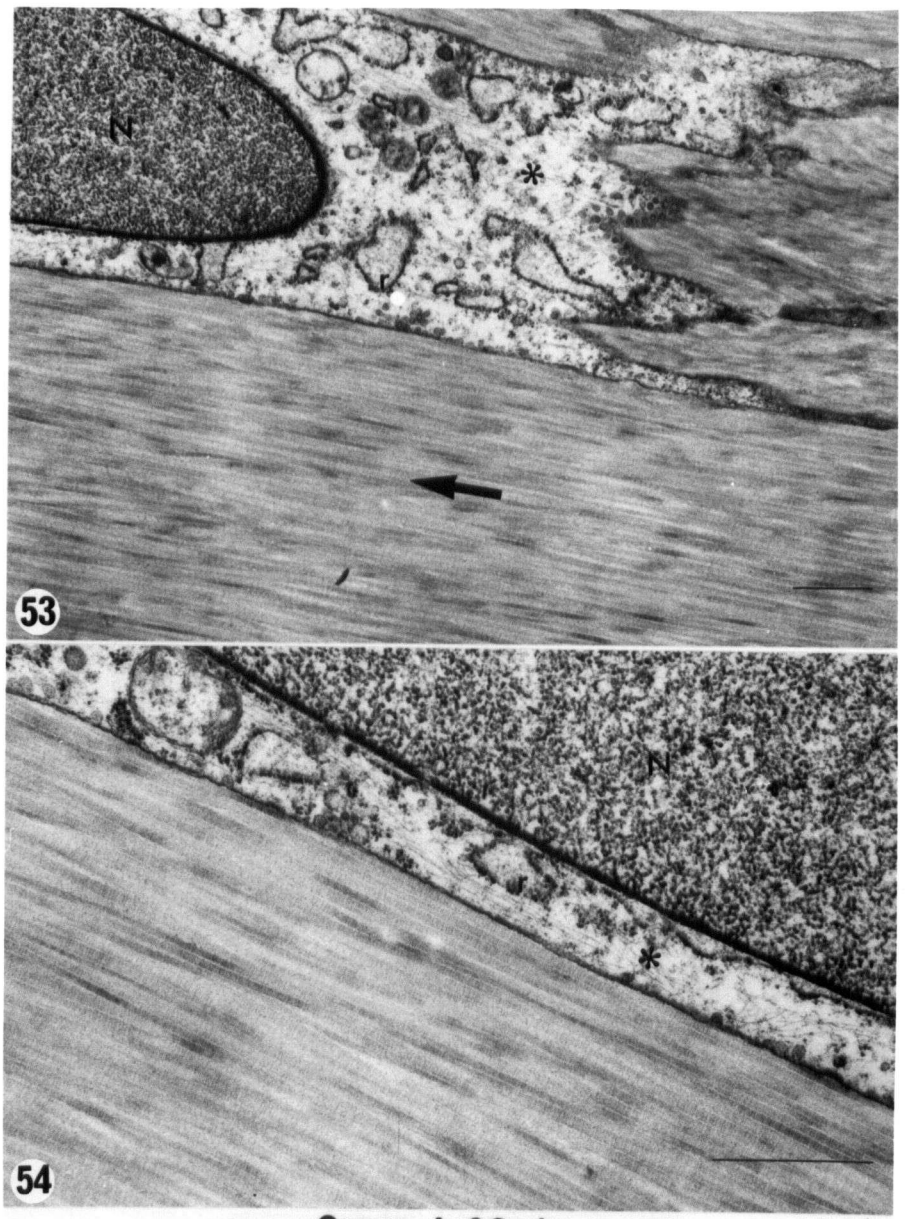
Figure 52: Electron micrograph of a repairing area of a ligament in Group D at 10 weeks post-operative. The metabolic activity of the fibroblast is reflected by the small numbers of mitochondria (m) and rough endoplasmic reticulum (r). Tropocollagen filaments are seen at the cell membrane (open arrow). A one micron bar marker is in the lower right corner. X 28,000.



Group D 10wks.

Figure 53: An electron micrograph of a repairing area of a ligament in Group A at 20 weeks post-operative. A small fibroblast is seen with a nucleus (N) and rough endoplasmic reticulum (r). There are only small amounts of intracellular organelles (asterix) as in controls. The collagen fibers are densely packed and longitudinal as indicated by the solid arrow. A one micron bar marker is in the lower right corner. X 14,300.

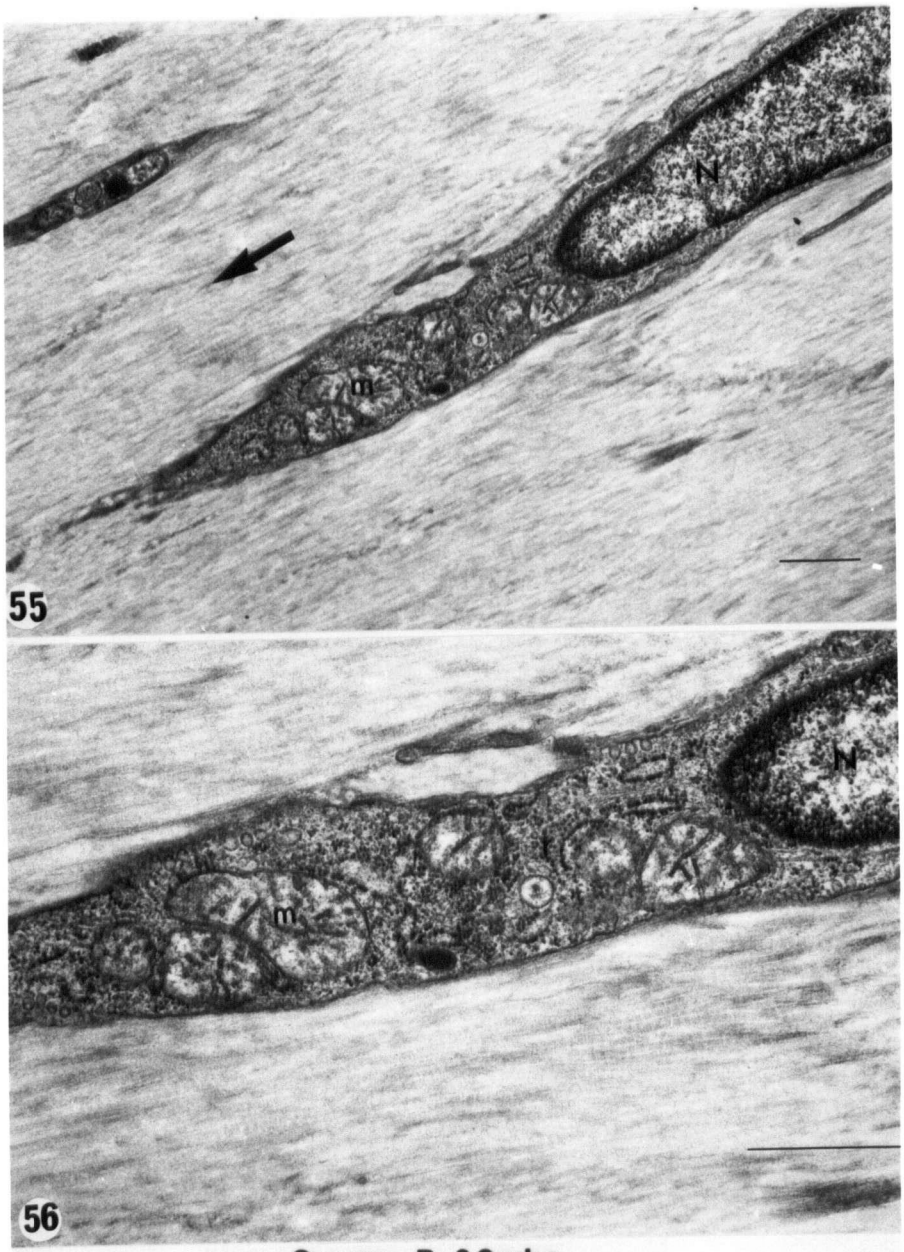
Figure 54: An electron micrograph of a repairing area of a ligament in Group A at 20 weeks post-operative. As in Figure 53 there are only small numbers of intracellular organelles (asterix) including rough endoplasmic reticulum (r). The collagen is densely packed and regular. A one micron bar marker is in the lower right corner. X 28,000.



Group A 20wks.

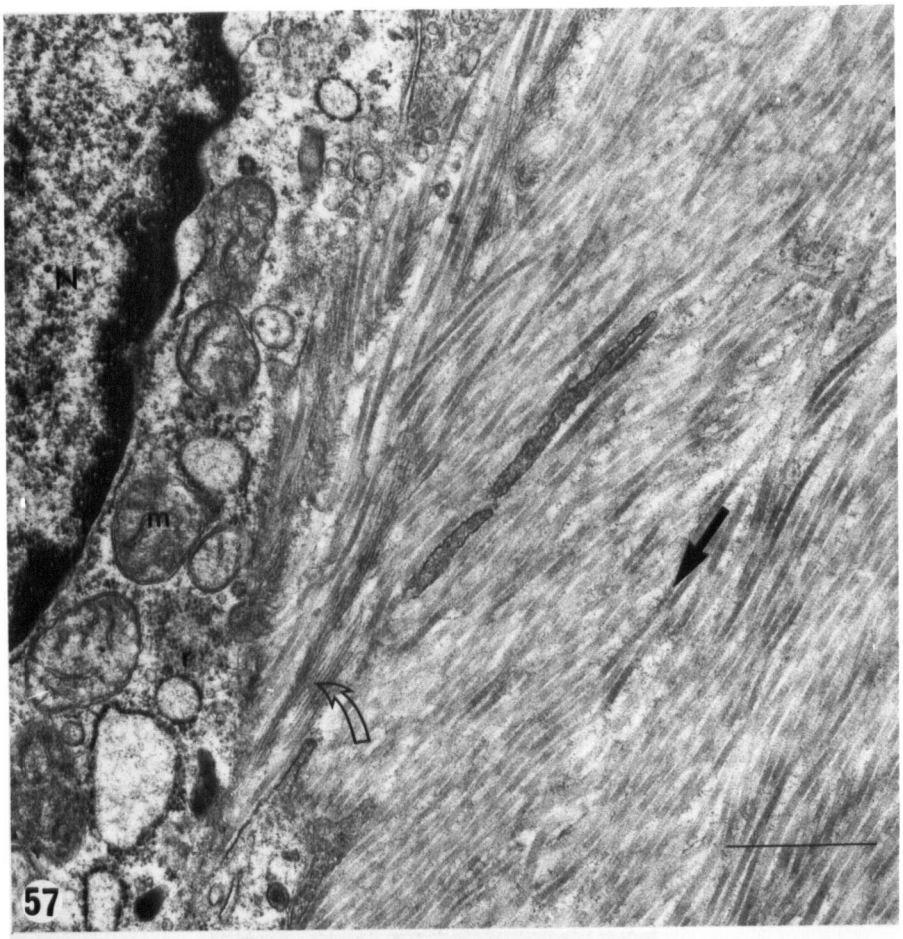
Figure 55: An electron micrograph of a repairing area of a ligament in Group B at 20 weeks post-operative. The fibroblasts are small but remain active metabolically with abundant mitochondria. The collagen is not as densely packed as in Group A but is mainly longitudinal as seen by the solid arrow. A one micron bar marker is in the lower right corner. X 14,300.

Figure 56: An electron micrograph of a repairing area of a ligament in Group B at 20 weeks post-operative at higher magnification. A metabolically active fibroblast is seen with abundant mitochondria (m) and rough endoplasmic reticulum (r). The collagen is not as densely packed as in Group A or controls. A one micron bar marker is in the lower right corner. X 28,000.



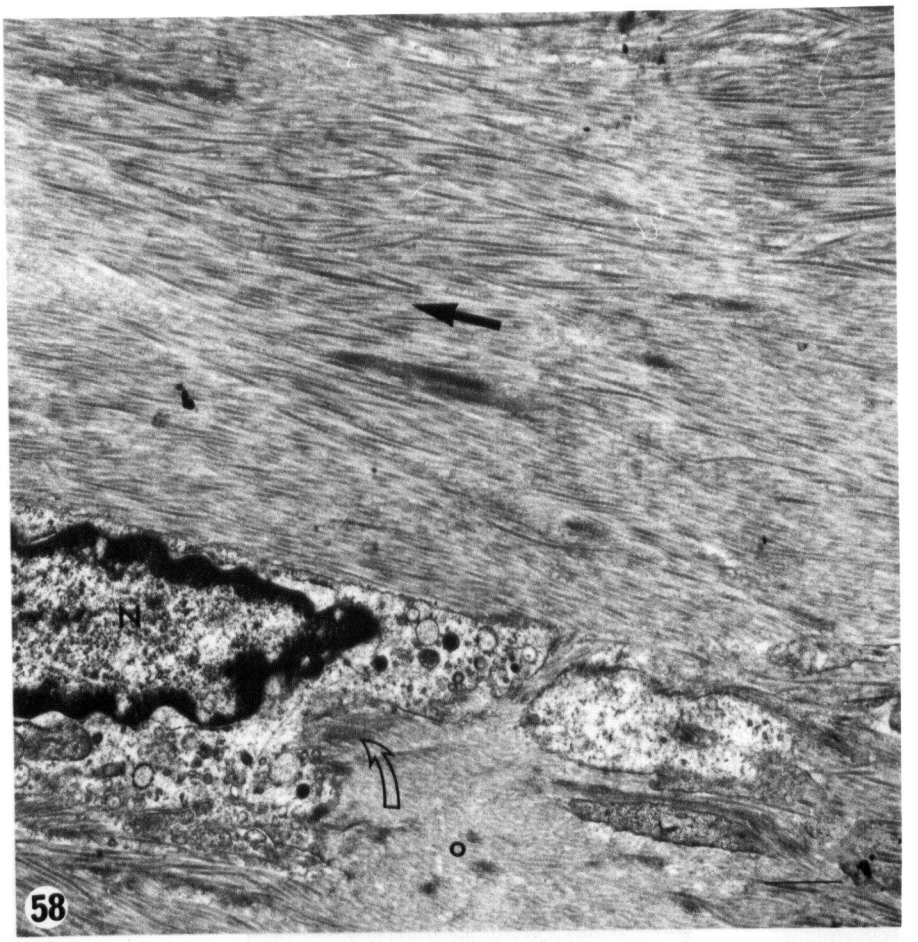
Group B 20wks.

Figure 57: An electron micrograph of a repairing area of a ligament in Group C at 20 weeks post-operative. The fibroblast is large with a large nucleus (N) and is still very active metabolically with abundant mitochondria (m) and rough endoplasmic reticulum (r). Tropocollagen filaments (open arrow) are seen at the cell margin. The collagen is more densely packed that at 10 weeks but still is not the same as controls and displays some branching. A one micron bar marker is in the lower right corner. X 28,000.



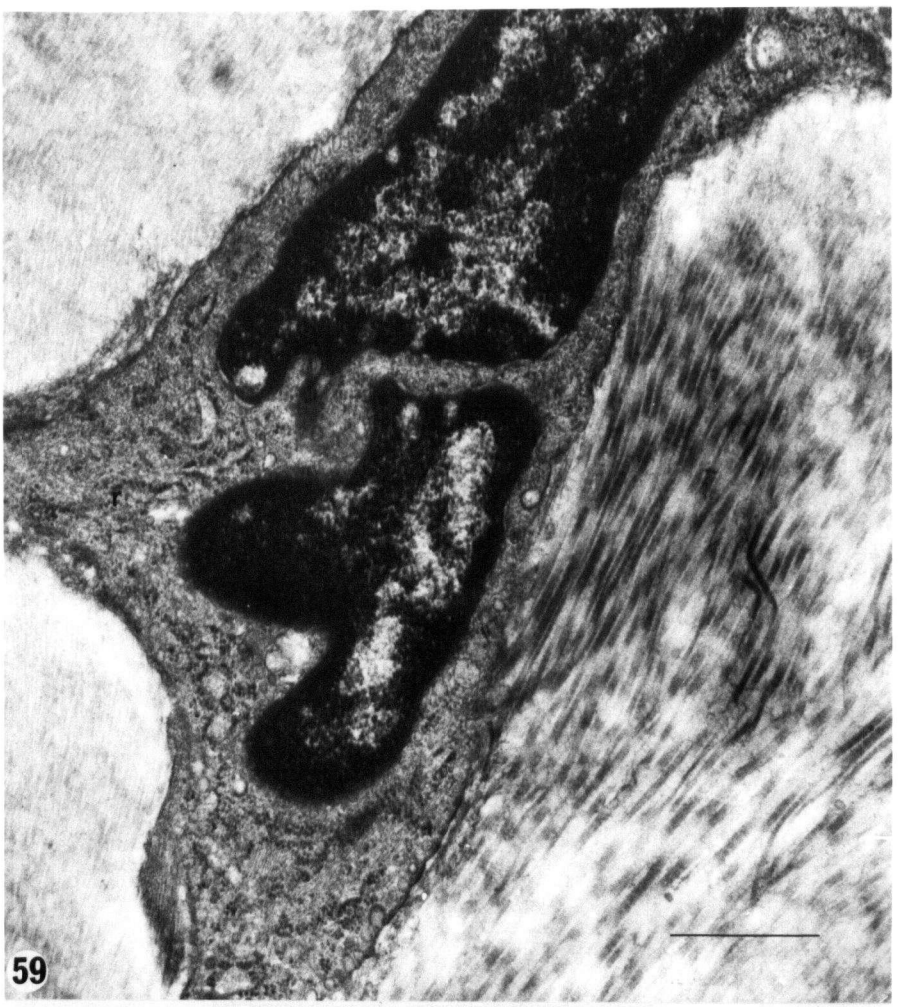
Group C 20wks.

Figure 58: An electron micrograph of a repairing area of a ligament in Group C at 20 weeks post-operative. A fibroblast with a large nucleus (N) is seen. This fibroblast is not as metabolically active but tropocollagen filaments (open arrow) are seen at the cell margin. The collagen fibers are similar to those seen in Figure 57 with branching seen and oblique fibers (o) in some portions. The longitudinal axis is indicated by the large solid arrow. A one micron bar marker is in the lower right corner. X 14,300.



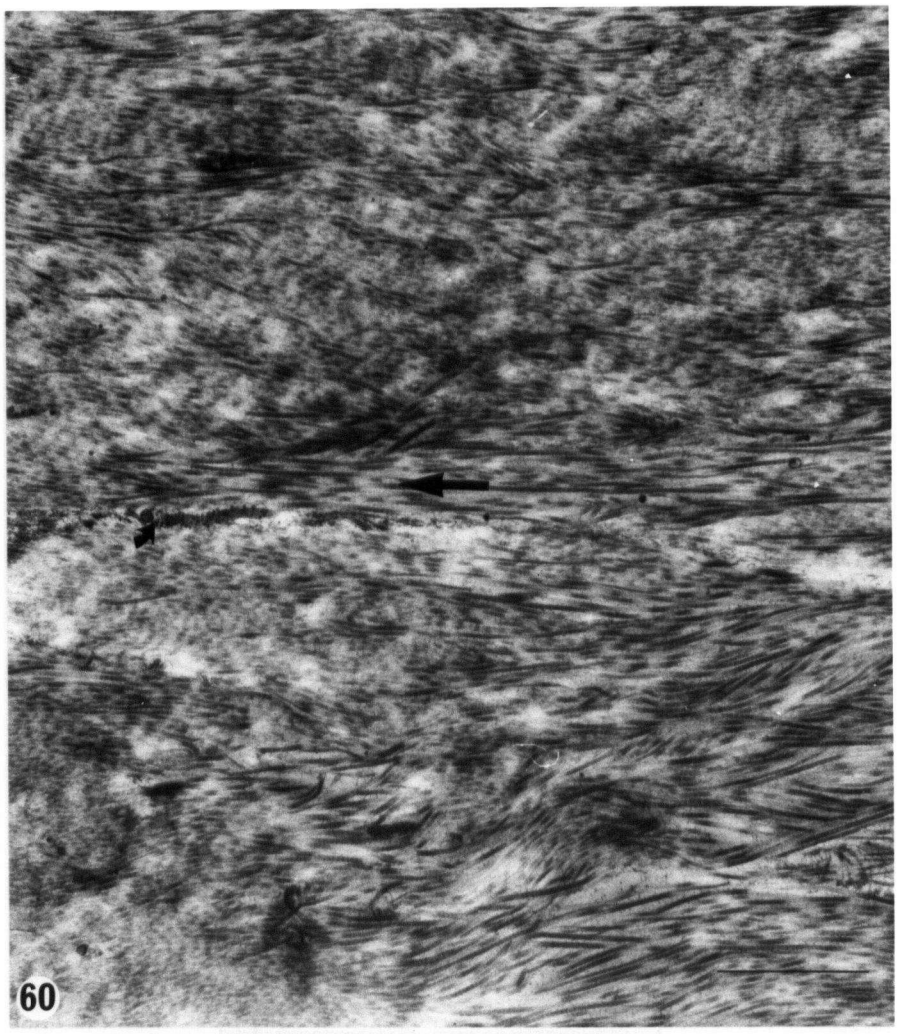
Group C 20wks.

Figure 59: An electron micrograph of a repairing area of a ligament in Group D at 20 weeks post-operative. A small fibroblast is seen and there is little metabolic activity with some rough endoplasmic reticulum (r) present. The collagen fibers are irregular and not as densely packed. A one micron bar marker is in the lower right corner. X 28,000.



Group D 20wks.

Figure 60: An electron micrograph of a repairing area of a ligament in Group D at 20 weeks post-operative. The collagen fibers mainly follow a longitudinal organization as indicated by the large solid arrow but irregularity and branching is still present. Extracellular debris (small solid arrow) is still present. A micron bar marker is in the lower right corner. X 28,000.



Group D 20wks.

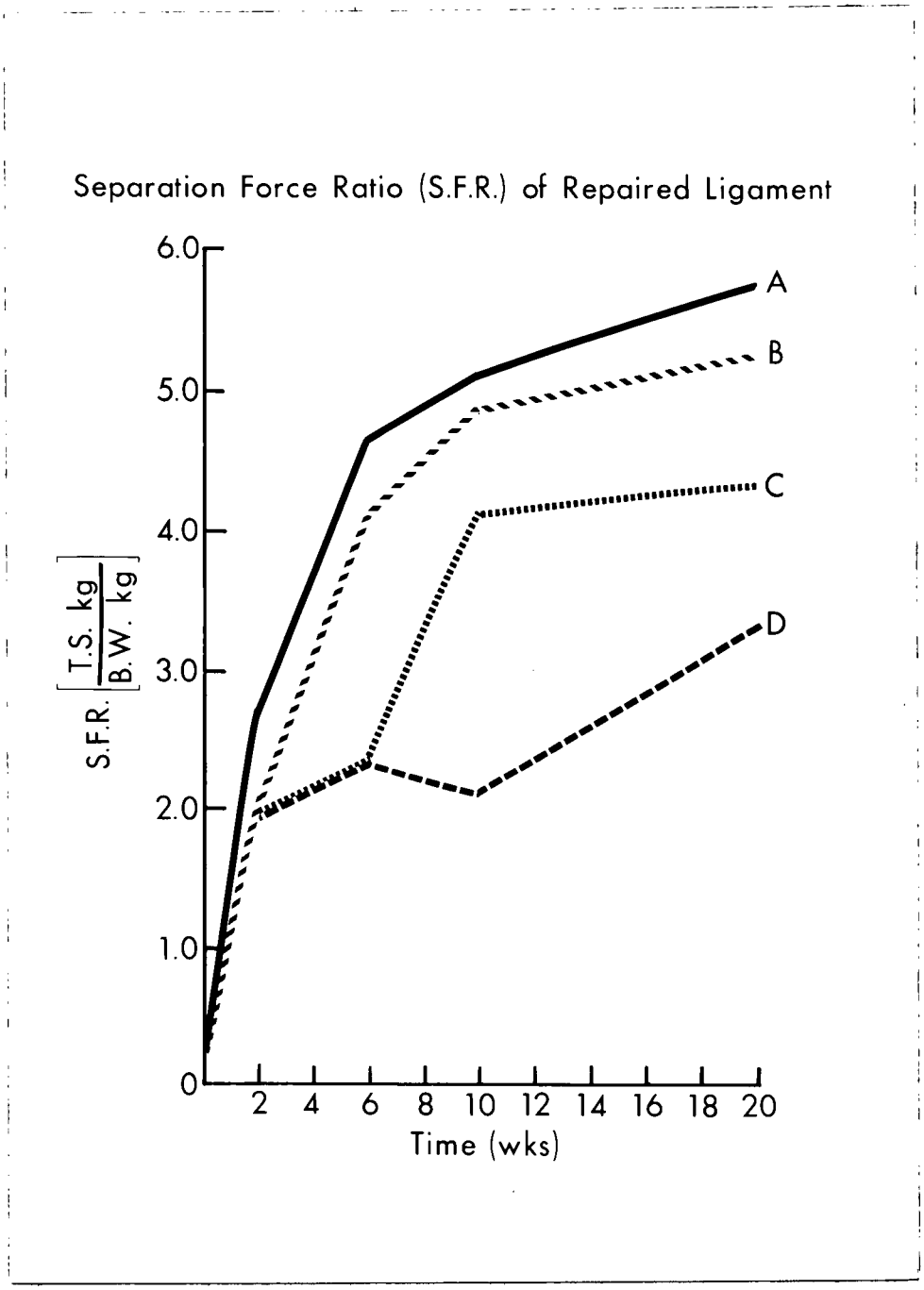


Figure 61: A graph of the Separation Force Ratio (see text) of the required ligament in each of the groups versus time (in weeks) post-operative. Group A had a significantly higher separation force ratio regaining its strength at a faster rate than the other groups.

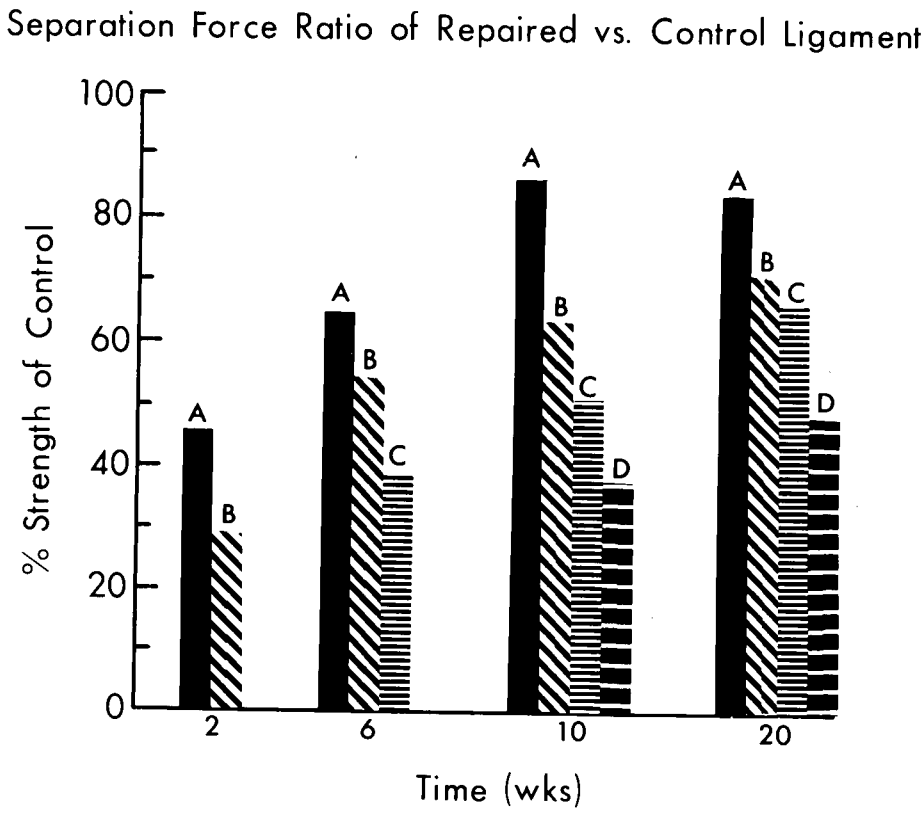


Figure 62: A bar graph of the Separation Force Ratio of the repaired ligament as compared to the control ligament versus time (in weeks) post-operative for each of the groups. Group A had a significantly higher separation force ratio and regained its strength at a faster rate than the other groups (see text). At 20 weeks post-operative Group A was 11% stronger than Group B, 32% stronger than Group C and 71% stronger than Group D.

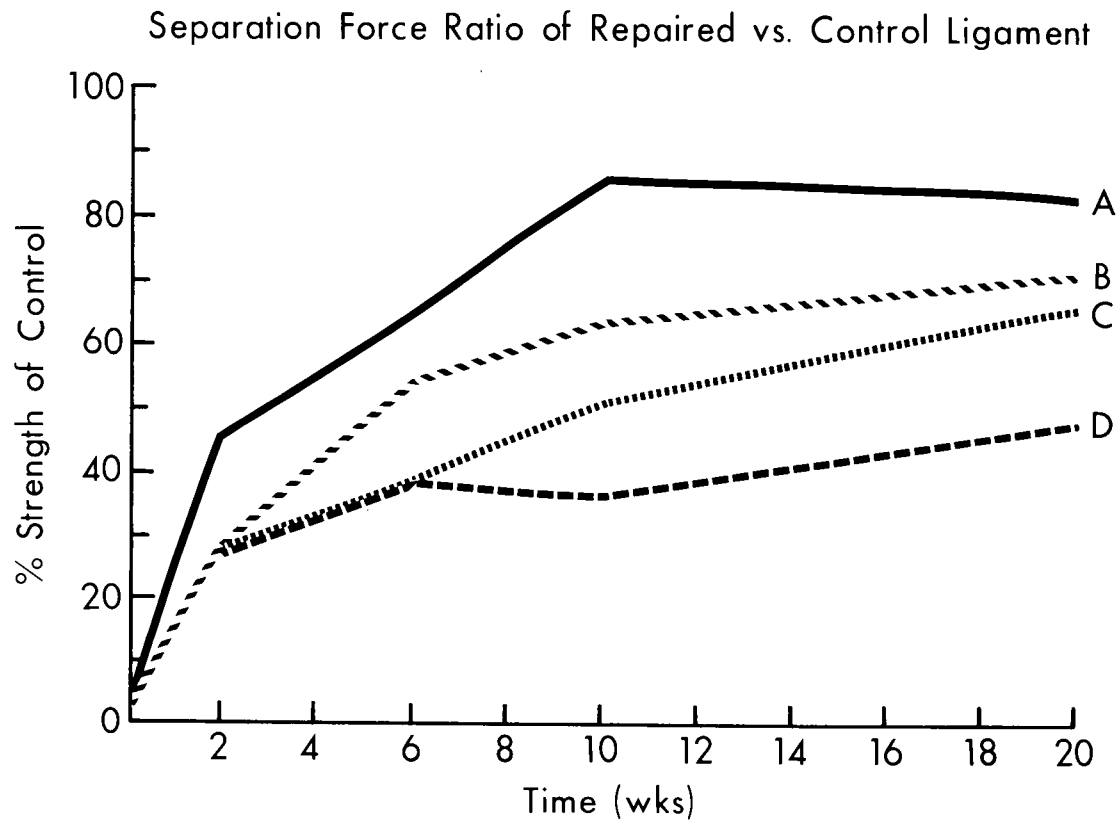


Figure 63: A line graph of the separation force ratio of the repaired ligament as compared to the control ligament versus time (in weeks) post-operative. The data is the same as in Figure 62.

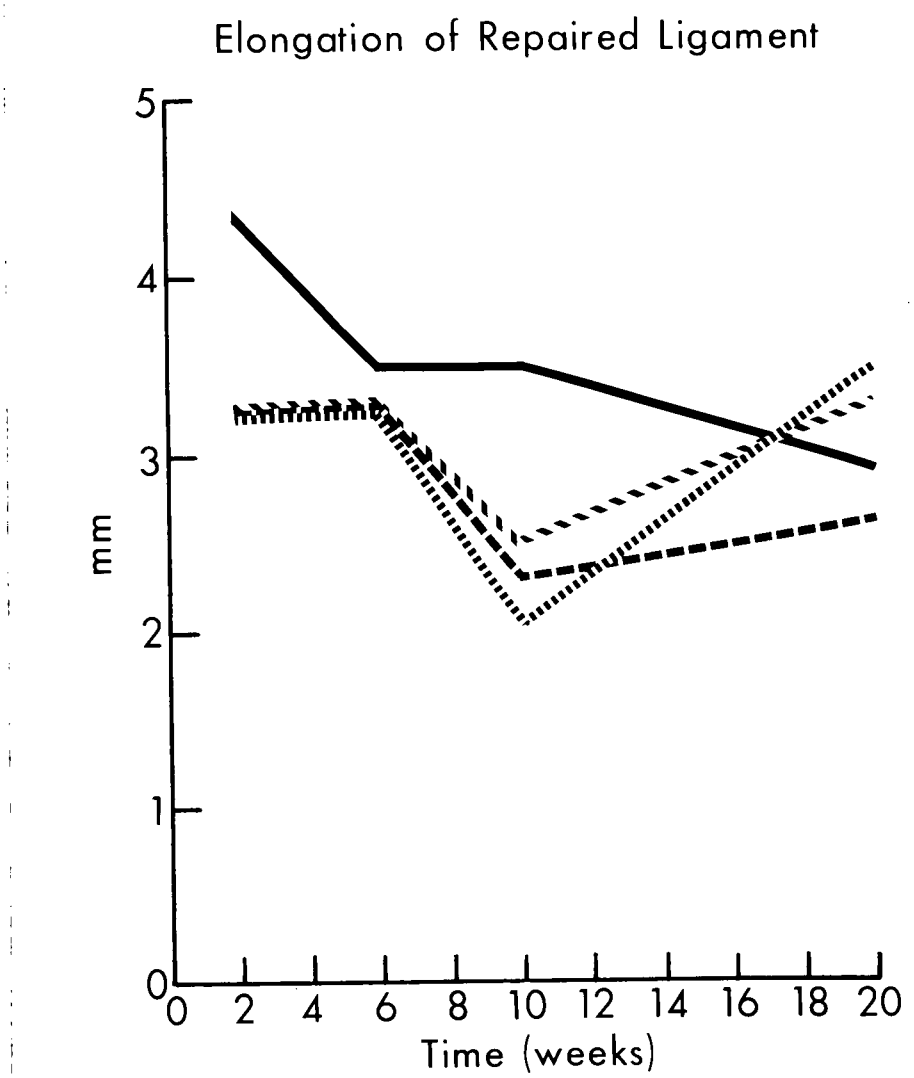


Figure 64: A graph of the elongation of the repaired ligament in millimeters versus time (in weeks) post-operative. No specific pattern could be derived and the differences between the versus groups was not significant.
Group A = Group B =
Group C = Group D =

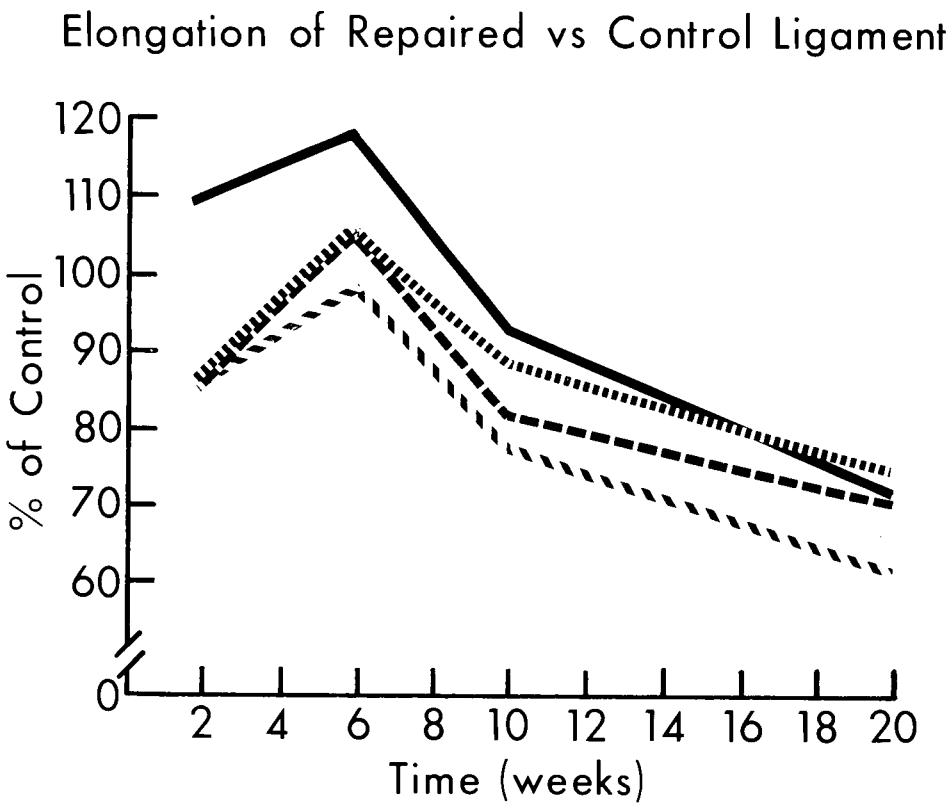


Figure 65: A graph of the elongation of the repaired ligament as compared to the control ligament versus time (in weeks) post-operative. All groups showed a peak at 6 weeks and decreased fdrom then on but there was no significant differences between the various groups.

Group A = Group B =

Group C = Group D =

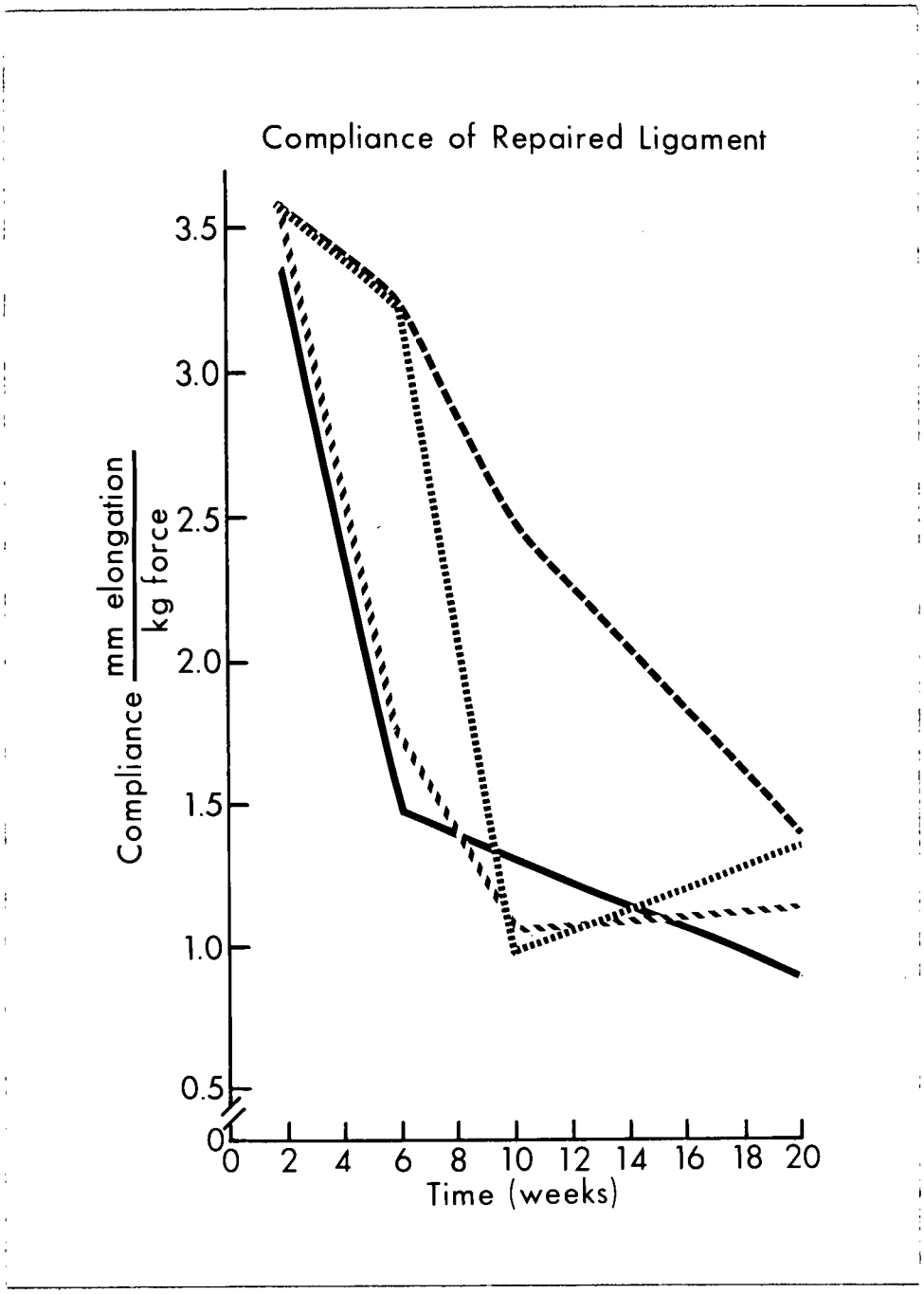


Figure 66: A graph of the compliance of the repaired ligament (see text) versus time in weeks post-operative. A trend in improvement is seen in all of the groups with no significant differences between the various groups.

Group A = Group B =

Group C = Group D =

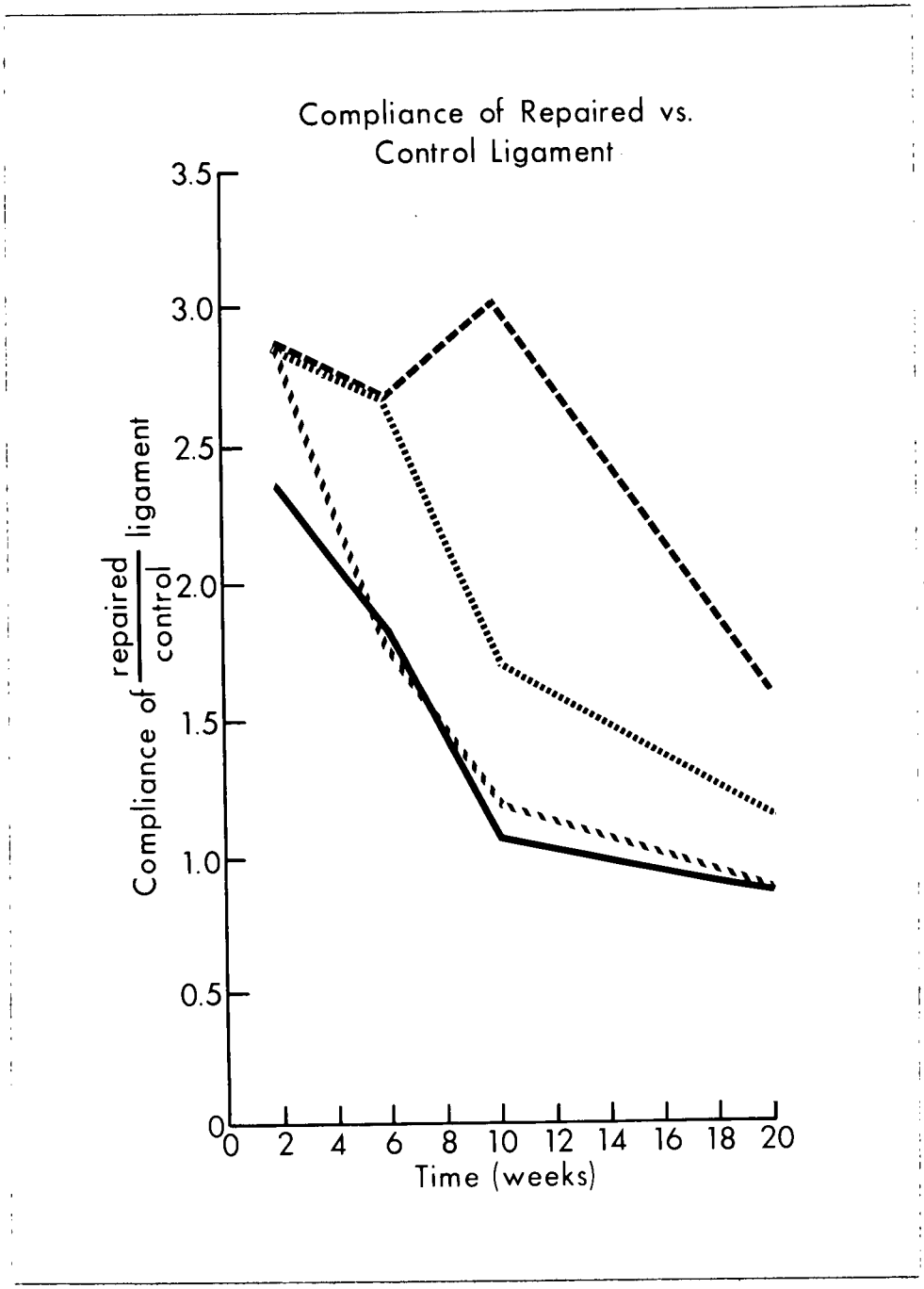


Figure 67: A graph of the compliance of the repaired ligament as compared to the control ligament, versus time (in weeks) postoperative. All groups showed an improvement in compliance with Groups A and B being significantly better than Groups C and D.
Group A = Group B =
Group C = Group D =

BIBLIOGRAPHY

1. Akeson, W.H. An Experimental Study of Joint Stiffness. J. Bone. Jt. Surg. 43A(7): 1022-1034.

2. Akeson, W.H. Connective Tissue Response to Immobility. Clin. Orthop. Rel. Res. 51: 194-197, 1967.

3. Akeson, W.H., Woo, S.L.Y., Amiel, D., et al. The Connective Tissue Response to Immobility: Biochemical Changes in Periarticular Connective Tissue of the Immobilized Rabbit Knee. Clin. Orthop. Rel. Res. 93: 356-362, 1973.

4. Alm, A., Ekstrom, H., Stromberg, B. Tensile Strength of the Anterior Cruciate Ligament in the Dog. Acta. Scan. Chir. Suppl. Vol. 445: 15-189.

5. Alm, A., Gillquist, J. Reconstruction of the Anterior Curciate Ligament By Using the Medial Third of the Patellar Ligament. Acta. Chir. Scand. 140: 289-296, 1974.

6. Alm, A., Liljedahl, S.O., Stromberg, B. Clinical and Experimental Experience in Reconstruction of the Anterior Cruciate Ligament. Orth. Clin. N.A. 7(1): 181-189, 1976.

7. Cabaud, H.E., Rodkey, W.G., Feagin, J.A. Experimental Studies of Anterior Cruciate Ligament Injury and Repair. Am. J. Sports Med. 7(1): 18-22, 1979.

8. Clayton, M.L., Weir, G.J. Experimental Investigation of Ligamentous Healing. Am. J. Surg. 98: 373-378, 1959.

9. Dehne, E., Torp, R.P. Treatment of Joint Injuries by Immediate Mobilization. Clin. Orthop. Rel. Res. 77: 218-232, 1971.

10. Evans, E.B., Eggers, G.W.N., Butler, J.K., Blumel, J. Experimental Immobilization and Remobilization of Rat Knee Joints. J. Bone. Jt. Surg. 42A(5): 737-758, 1960.

11. Forward, A.D., Cowan, R.J. Tendon Suture to Bone. J. Bone Jt. Surg. 45A(4): 807-822, 1963.

12. Grant, M.E., Prockop, D.J. The Biosynthesis of Collagen. New Eng. J. Med. 286: 194-199, 242-249, 291-300, 1972.

13. Greene, E.C. Anatomy of the Rat. Vol. 27, Hofner Publishing Co. N.Y. 1955, 375 pp.

14. Haggmark, T., Erikson, E. Cylinger or Mobile Cast Brace after Knee Ligament Surgery. Am. J. Sports Med. 7(1): 48-56, 1979.

15. Hirsch, G. Tensile Properties During Tendon Healing. Acta. Orthop. Scand. Suppl. No. 153: 8-79, 1974.

16. Ippolito, E., Natali, P.G., Postucchini, F., et al. Morphological, Immunochemical and Biochemical Study of Rabbit Achilles Tendon at Various Ages. J. Bone Jt. Surg. 62A(4): 583-597, 1980.

17. Jack, E.A. Experimental Rupture of the Medial Collateral Ligament of the Knee. J. Bone Jt. Surg. 32B(3): 396-402, 1950.

18. Kappakas, G.S., Brown, T.D., Goodman, M.A., et al. Delayed Surgical Repair of Ruptured Ligaments: A Comparative Biomechanical and Histological Study. Clin. Orthop. Rel. Res. 135: 281-286, 1978.

19. Klein, L., Lewis, J. Simultaneous Quantification of H-Collagen Loss and H. Collagen Replacement During Healing of Rat Tendon Grafts. J. Bone Jt. Surg. 54A(1): 137-146, 1972.

20. Laros, G.S., Tipton, C.M., Cooper, R.R. Influence of Physical Activity on Ligament Insertions in the Knees of Dogs. J. Bone Jt. Surg. 53A(2): 275-286, 1971.

21. Lindsay, W.K., Thomson, H.G. Digital Flexor Tendons: An Experimental Study. Br. J. Plastic Surg. 12: 289-319, 1960.

22. Mason, M.L., Allen, H.S. The Rate of Healing of Tendons. Annals of Surgery 113(3): 424-459, 1941.

23. Noyes, F.R., DeLucas, M.S., Torvik, P.J. Biomechanics of Anterior Cruciate Ligament Failure: An Alysis of Strain-Rate Sensitivity and Mechanisms of Failure in Primates. J. Bone Jt. Surg. 56A(2): 236-253, 1974.

24. Noyes, F.R., Torvik, P.J., Hydo, W.B. Biomechanics of Ligament Failure. J. Bone Jt. Surg. 56A(7): 1406-1418, 1974.

25. Noyes, F.R., Grood, E.S. The Strength of the Anterior Cruciate Ligament in Humans and Rhesus Monkeys. J. Bone Jt. Surg. 58A(8): 1074-1082, 1976.

26. Noyes, F.R. Functional Properties of Knee Ligaments and Alterations Induced by Immobilization. Cl. Orth. Rel. Res. 123: 210-242, 1977.

27. O'Donoghue, D.H., Rockwood, C.A., Zaricznys, B., Kenyon, R. Repair of Knee Ligaments in Dogs. J. Bone Jt. Surg. 43A(8): 1167-1178, 1961.

28. O'Donoghue, D.H., Rockwood, C.A., Frank, G.R., et al. Repair of the Anterior Cruciate Ligament in Dogs. J. Bone Jt. Surg. 48A(3): 503-519, 1966.

29. O'Donoghue, D.H., Gael, R.F., Jeter, G.L., et al. Repair and Reconstruction of the Anterior Cruciate Ligament in Dogs. J. Bone Jt. Surg. 53A(4): 710-718, 1971.

30. Perkins, G. Rest and Movement. J. Bone Jt. Surg. 35B(4): 521-539, 1953.

31. Ross, R. The Fibroblast and Wound Repair. Biol. Rev. 43: 51-96, 1968.

32. Salter, R.B., Field, P. The Effects of Continuous Compression on Living Articular Cartilage. J. Bone Jt. Surg. 42A(1): 31-49, 1960.

33. Salter, R.B., Simmonds, D.F., Malcolm, B.W., et al. The Biologic Effect of Continuous Passive Motion on the Healing of Full Thickness Defects in Articular Cartilage. J. Bone Jt. Surg. 62A(8): 1232-1251, 1980.

34. Tipton, C.M., Schild, R.J., Flatt, A.E. Measurement of Ligamentous Strength in Rat Knee. J. Bone Jt. Surg. 49A(1): 63-72, 1967.

35. Tipton, C.M., Schild, R.J., Tomanck, R.J. Influence of Physical Activity on the Strength of Knee Ligaments in Rats. J. Physiol. 212(4): 783-787, 1967.

36. Woo, S.L-Y., Matthews, J.V., Akeson, W.H., et al. Connective Tissue Response to Immobility. Arthritis and Rheumatism 18(3): 257-264, 1975.

37. Zingg, W. Bioengineering Analysis of Healing Tissues. J. Sports Med. 3(2): 61-70, 1975.

38. Zuckerman, J., Stull, G.A. Effects of Exercise on Knee Ligament Separation Force in Rats. J. Appl. Physiol. 26(6): 716-719, 1969.