

Autogenous Transplantation of Rib Cartilage Preserved in Glycerol, after Removal of the Perichondrium, to the Malar Process of Rats

—A Histological Study (PART I)—

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(Received 13 February and accepted 22 February 1990)

Key words: preserved cartilage, glycerol, transplantation

Abstract

Seventy-two male albino rats received autogenous transplants of glycerol-preserved rib cartilage into the malar process. The animals were divided into two groups which received preserved cartilage with or without perichondrium. The implants were well tolerated and removal of the perichondrium enhanced the rate of resorption and bone replacement of the material.

Introduction

Fresh transplanted cartilage retains its cell viability through a normal mechanism of nutrition, involving osmotic diffusion of interstitial fluid from the host bed^[1] before penetration by newly formed capillaries. With regard to its structural and nutritional characteristics, cartilage is very well suited for transplantation procedures, with a high level of anaerobic metabolism enabling it to survive anoxic periods^[2].

Fresh homogeneous cartilage grafts are noted for their long-term cell survival rate, which has been linked to a possible protective action of the matrix, either mechanical or chemical^[3,4]. As a consequence of long-term survival, resorption and bone substitution of fresh cartilage grafts are slow and limited^[1]. Autogenous grafts also show a tendency to grow^[5], although perichondrial removal promotes faster and more intense resorption and substitution by bone tissue^[6].

Several methods have been used to preserve cartilage, such as lyophilization^[7], freeze-drying^[8], immersion in unsaturated polyester^[9], immersion in alcohol^[10],

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This study was performed with a grant from FAPESP and presented with the National Dentistry Award "Horace Wells-87" (*National Council of Dentistry-Merrel-Lepetit*).

immersion in a solution of merthiolate and saline^[11], boiling and freezing in CO₂^[6], refrigeration^[12], immersion in paraffin^[9], immersion in Ringer solution^[13] and autoclaving^[14].

Because of the subtle immunological properties of cartilage, methods for its preservation should not include drastic chemical treatments. For this reason, more biological methods, such as freezing or freeze-drying, are now used^[12]. The material thus treated seems to be more rapidly revascularized, resorbed and remodeled than if preserved by more aggressive methods, such as boiling or enzymatic inactivation^[12].

Cryogenic methods, although superior to chemical treatment, require special equipment for storage and transportation. Glycerol, which is widely used as a cryoprotectant for preserving the viability of tissues to be treated by deep-freezing and drying, seems to remove most of the intracellular water, without altering the intracellular ionic composition. Thus, in these procedures, glycerol acts as a very efficient protector of cellular integrity^[15].

At higher concentrations, as used by PIGOSI^[15] to preserve homogeneous duramater, and by KING, McTIGUE and MERYNAN^[16] in the preservation of corneas, glycerol maintains the integrity and texture of the preserved material^[17-19], reduces its antigenic properties and promotes sterilization^[6], although, of course, such high concentrations of glycerol devitalize the preserved tissue^[15,16]. This is a simple and inexpensive method, and storage and transportation can be done at room temperature.

Autogenous cartilage preserved in glycerol shows more intense new bone formation than fresh tissue, with comparable degrees of inflammatory reaction^[20]. The present study was designed to verify whether bone neoformation and graft substitution could be further enhanced by transplantation of autogenous cartilage preserved in glycerol after removal of the perichondrium.

Materials and Methods

In the present investigation, 72 male albino rats (*Rattus norvegicus*, *albinus*, Wistar) weighing from 300 to 350 g were used. The animals were fed before and during the experiment with solid food (Ração Ativada "Produtor", Anderson Clayton S/A), except for the first 24 h postoperatively, and were supplied water *ad libitum*.

1. REMOVAL OF COSTAL CARTILAGE

Under general anesthesia, induced through intraperitoneal infusion of thionembutal (Abott Laboratórios do Brasil Ltda) at an approximate dose of 50 mg/kg body weight, the left thoracic and infra orbital regions were depilated.

With the animal positioned ventral side uppermost on a surgical table, the operative fields were disinfected with merthiolate solution (Lilly), and access to the last rib was obtained by means of a skin incision and divulsion of the subcutaneous tissues, using medium straight scissors.

From the free end of the last (13th) rib, a terminal segment measuring about 5 mm in length was removed, and the wound sutured with 5-0 polyvicryl thread (Ethicon). The removal of the rib segment was best achieved when the rib was

isolated and pulled upwards, allowing removal of fascia debris before the rib was cut. Other fragments of tissue adhering to the segment were carefully eliminated later using a scalpel, without damaging the perichondrium or cartilage.

2. *GRAFTING PROCEDURE*

With the animal lying on its right side, surgical access to the malar process of the maxilla was obtained through an incision, using a scalpel fitted with a No. 15 blade, running about 2 mm below the inferior border of the left orbital floor, following its contour, with an approximate length of 10 mm.

After divulsion of the soft tissues using small acute-ended scissors, the periosteum was also incised and reflected by means of a No. 7 wax spatula and a No. 2 Holleback instrument. The soft tissues were held in position by means of two iridectomy nippers.

Next, using a No. 702 tapered burr (Maillefer, Switzerland) mounted on a low-speed drill, a bone cavity was prepared on the malar process. This osteotomy was about 4 mm long and 2 mm deep, the width being slightly more than the burr diameter. The cavity was drilled into the cortical and cancellous bone, avoiding perforation of the malar process, and keeping within its limits. During the whole procedure, saline was used for refrigeration as well as removal of bone debris.

After adapting the graft to the osseous bed (Fig. 1) using iridectomy nippers, the periosteum was returned to its original position, covering the transplanted material, and the deep and superficial planes were sutured with 5-0 polyvicryl thread.

Immediately afterwards, each animal received an intraperitoneal infusion of 16,000 U. I. of Penicillin G Benzatin (Benzetacil K. Fontoura Wyeth).

3. *EXPERIMENTAL GROUPS*

All the animals underwent the same surgical procedures, but were divided into the following two groups according to whether or not the perichondrium was removed.

GROUP I: After removal, the cartilage was washed for 15 min with saline and maintained for 20 days in 98% glycerol (Química Moura-Brasil). Just prior to implantation, it was rehydrated for 5 min in saline. All the animals had been identified previously, thus permitting the use of autogenous material. This method of preservation corresponded to that proposed by PIGOSI^[15] for the storage of homogeneous duramater, with a few adaptations due to the dimensional characteristics of the implant used.

GROUP II: Before preservation in glycerol, the perichondrium was removed from all grafts by securing one of the extremities with iridectomy nippers and scraping with No. 11 and 15 blades, under magnification.

4. *REMOVAL OF THE SPECIMENS FOR HISTOLOGICAL PROCESSING*

Six animals in each group were sacrificed after 5, 10, 20, 30, 60 and 120 days, postoperatively, by inhalation of sulfuric ether, and the whole zygomatic arch of each animal was removed.

In order to perform this procedure, a cut was made with medium scissors along the long axis of the cranium and the arch was dissected out using scalpels fitted with both No. 11 and 15 blades and also acute-ended scissors. Osteotomies

for removing the whole arch were performed using medium curved scissors.

The specimens were fixed in 10% formalin, and decalcified in 50% sodium citrate/formic acid solution, following routine laboratory procedures.

The blocks of tissues were cut semiserially into 6- μ m-thick sections, which were then stained with hematoxylin/eosin for histological analysis.

Results

5 days

GROUP I: The cartilage held its position in the host bed, without evidence of resorption. Adjacent to the material, besides blood clots, moderate numbers of neutrophils and numerous histiocytes were seen. In areas located further away from the cartilage, intense fibroblast proliferation and newly formed capillaries were found. A moderate number of lymphocytes was also present in this region.

GROUP II: The cartilage without perichondrium was maintained in position within the host bed without signs of resorption. Closer to the material, characteristics of inflammatory infiltration were comparable to those observed in the previous group. However, in areas further away from the material, there was no evidence of connective tissue neoformation. A large amount of inflammatory exudate was present as well as intense inflammatory infiltration, with predominance of neutrophils.

10 days

GROUP I: In almost all specimens, well vascularized newly formed connective tissue, with a moderate number of fibroblasts, was seen adjacent to the perichondrium (Fig. 2).

Discrete inflammatory infiltration was seen in this region with predominance of lymphocytes. In areas further away, connective tissue rich in collagen fibers was observed.

GROUP II: The cartilage without perichondrium was in direct contact with newly formed connective tissue (Fig. 3) rich in collagen fibers.

In one of the specimens, the material was partly resorbed and substitution by newly formed connective tissue was seen.

20 days

GROUP I: In most specimens, intense bone neoformation was found adjacent to the material, so that between the bone and perichondrium, only a thin connective tissue lining was seen (Fig. 4). In two specimens, however, bone neoformation was not as intense, and a thick connective tissue lining was evident, with discrete lymphocytic inflammatory infiltration.

GROUP II: In the group without perichondrium, three specimens showed extensive resorption of the cartilage, with newly formed bone in close contact with the remainder of the graft (Fig. 5).

In one of the specimens, only a small portion of the graft was found. In the other specimens, however, the transplanted cartilage was surrounded by connective tissue infiltrated by a few lymphocytes. The resorption was only obvious in these instances, and at some points newly formed bone was evident inside the graft.

30 days

GROUP I: In all specimens, new bone formation was intense, about 2/3 of the host bed being repaired by bone. At several points, the newly formed bone and graft were separated by perichondrium and newly formed fibrous connective tissue (Fig. 6). At other sites, however, cartilage was partly resorbed and substitution by newly formed bone occurred, especially where the perichondrium was absent. In these instances, the bone was in intimate contact with the remaining cartilage.

GROUP II: In the specimens without perichondrium, extensive resorption of cartilage was observed in most cases. In one specimen, only a small portion of the transplanted material was found. In others, greater amounts of cartilage were still present, partly replaced by newly formed bone. Extensive areas of bone were in direct contact with the remaining cartilage (Fig. 7).

60 days

GROUP I: In most cases, the host bed was practically filled with newly formed bone around the transplanted cartilage. At several sites, however, where perichondrium was present, a space existed between the bone and cartilage, filled with fibrous connective tissue (Fig. 8). At other locations the graft, partly resorbed, was replaced by bone.

GROUP II: In most cases, the cartilage without perichondrium showed the same degree of resorption as that in the previous stage for the same group. However, newly formed bone was in contact with the remaining cartilage throughout its whole extent (Fig. 9).

120 days

GROUP I: The cartilage at some points showed areas of resorption filled with newly formed bone. On the other hand, where the perichondrium persisted, a layer of connective tissue separated it from the bone (Fig. 10). At locations where the perichondrium was not present, the connective tissue was in close contact with the cartilage.

GROUP II: The remaining cartilage was totally occupied by newly formed bone (Fig. 11). In several places, the bone trabeculae filled the spaces created by resorption of cartilage. The amount of remaining cartilage was comparable to that observed at the initial stage in the same group. In one specimen, the cartilage was almost totally resorbed.

Discussion

In this study, autogenous cartilage was used to evaluate the effects of the preservation method, without possible immunological biases. Although cartilage is only mildly immunogenic, chondrocytes do possess superficial antigens^[21].

The malar process is a vascular host bed where osteogenic repair can occur, and thus it is suited for implantation of devitalized tissue, since this type of material does not "take" well at sites where this process is not normally observed^[22].

In both groups, the inflammatory reaction evident after 5 days resulted not only from the presence of the transplanted material, but also reflected the initial stages of healing. However, the presence of perichondrium seems to have favored

an earlier start to fibroblastic proliferation around the implanted material.

On the other hand, perichondrium delays the resorption of grafted material, which started after 10 days in specimens from which it had been removed. In fact, the presence of perichondrium delayed resorption and bone replacement of fresh autogenous grafts, whereas in the absence of perichondrium, faster resorption and replacement was seen in areas where the rib had been sectioned during harvesting^[20]. This behavior is probably due to the fact that the perichondrium, whether devitalized or not, may function as a fibrous barrier to resorption, and may stimulate the development of a fibrous capsule and isolation of the implanted material.

After 20 days, several specimens from which the perichondrium had been removed showed extensive resorption of the cartilage, and after 30 days their tendency to show faster replacement by bone was confirmed. The ossification of fresh autogenous cartilage grafts transplanted to the malar process of rats, as suggested by their vitality after 120 days^[5,6,20], may occur endochondrally^[4,8], being slower than replacement of devitalized tissue^[20].

The results of the present study showed that after 30 days, faster ossification occurred in the implants without perichondrium compared with rates observed previously for fresh and preserved grafts with perichondrium in the same region^[5,6,20].

After 60 and 120 days, the cartilage without perichondrium showed more resorption than corresponding grafts in the other group. At these stages, the perichondrium, in some areas, was still a barrier to osseous replacement, and a newly formed connective tissue lining still existed. Since the implants were autogenous, eliminating the possibility of an immune reaction, the perichondrium possibly constitutes a physical barrier due to its structural characteristics.

After 30 days, the rate of resorption slowed in both groups. This effect was most evident in group II. It seems that from this stage onwards, the rate at which the host replaces the material was reduced, possibly due to reduction in the quantity of cartilage present to a level tolerable to the host, as well as the excellent biocompatibility of the material. The total resorption suggested by TULIO^[5,6] to occur within 120 days, may be delayed due to host tolerance to the implanted material.

Studies on the removal of perichondrium and preservation in glycerol are now in progress using homogeneous material to determine whether even faster bone replacement can be induced by reduction of antigenicity.

Conclusions

Within the limits of the experimental conditions used in this study, we conclude that:

1. Preservation in glycerol maintained the structure of the cartilage, and the preserved material was well tolerated as an intra-osseous implant.
2. The resorption of cartilage lacking perichondrium was faster and more intense than that observed in the control group.
3. Bone neoformation was faster and more intense in implants without perichon-

drium.

4. The use of cartilage lacking perichondrium, and preserved in glycerol, seems to have potential for oral surgery.

References

- [1] PEER, L. A.: *Transplantation of Tissues. Cartilage, Bone, Fascia, Tendon and Muscle*, The Williams and Wilkins Co., Baltimore, USA, p. 421, 1955
- [2] LASKIN, D. M. and SARNAT, B. G.: The metabolism of fresh, transplanted and preserved cartilage, *Surg. Gynecol. Obstet.*, **96**, 493-499, 1953
- [3] KRAJINIK, VON J., LAZOWSKI, J., JACHIMOWICZ, B. and RAKOKSKI, J.: On the treatment of mandibular fracture with inflammatory complication in the fracture region by means of xenogenous cartilagenous tissue, *Stomatol. DDR.*, **28**, 268-274, 1978
- [4] PEACOCK JR., E. C., WEEKS, P. M. and PETTY, J. M.: Some studies on the antigenicity of cartilage, *Ann. N. Y. Acad. Sci.*, **87**, 175-183, 1960
- [5] TULIO, S.: *Enxerto autógeno livre de cartilagem hialina em mandíbula de ratos. Estudo histológico*, Araçatuba, Faculdade de Odontologia de Araçatuba, UNESP, 1982/Tese/
- [6] TULIO, S.: *Enxerto autógeno livre de cartilagem hialina com e sem a remoção do pericôndrio, em processo malar de ratos. Estudo histológico*, Araçatuba, Faculdade de Odontologia de Araçatuba, UNESP, 1984/Tese/
- [7] BROWN, L. K. B. and CRUESS, R. L.: Bone and cartilage transplantation in orthopaedic surgery. A review, *J. Bone Joint Surg.*, **64**, 270-279, 1982
- [8] HAM, A. W. and LEESON, T. S.: *Tratado de Histologia*, 7th ed., México, Interamericana, p. 935, 1975
- [9] IMMALIEV, A. S., VISHNEVETSKII, E. F., DAYKITES, A. I. and ABZHILLOV, M. A.: Biological properties of allogeneic cartilage preserved in paraffin and its utilization in reconstructive otorhinolaryngology, *Acta clin. Plast.*, **21**, 105-113, 1979
- [10] PEER, L. A.: The fate of living and dead cartilage transplanted in humans, *Surg. Gynecol. Obst.*, **68**, 603-610, 1939
- [11] DUPERTUIS, S. M.: Actual growth of young cartilage transplants in rabbits. Experimental studies, *Arch. Surg.*, **43**, 32-63
- [12] BRENT, B.: The versatile cartilage homograft: current trends in clinical transplantation, *Clin. plast. Surg.*, **6**, 163-180, 1979
- [13] LYE, T. L.: A histologic evaluation of cartilage homograft used in preprosthetic surgery, *Oral Surg.*, **31**, 745-763, 1971
- [14] GOODE, R. L.: Bone and cartilage graft: current concepts, *Otolaringol. Clin. North Am.*, **5**, 447-451, 1973
- [15] PIGOSI, N.: Implantação de dura-mater homogênea conservada em glicerina. Estudo experimental em cães, *Arg. Cir. Exper.*, **27**, 213-247, 1964
- [16] KING JR., J. H., MCTIGUE, J. W. and MERYMAN, H. T.: A simple method of preservation of corneas for lamellar keratoplasty, *Am. J. Ophthalmol.*, **53**, 445-449, 1962
- [17] PUIG, L. B., VERGINELLI, G., KAWABE, L. and ZERBINI, E. J.: Valva cardíaca de dura-mater homóloga. Método de preparação da valva, *Rev. Hosp. Cline. Fac. Med. S. Paulo*, **29**, 85-89, 1974
- [18] STOLF, N. A. G. and ZERBINI, E. J.: Correção de hérnia diafragmática utilizando dura-mater homóloga conservada em glicerina, *Rev. paul. Med.*, **83**, 21-24, 1974
- [19] ZERBINI, E. J. and PUIG, L. V.: Valvas cardíacas e biológicas, com especial referência à valva de dura-máter, *Arch. Inst. Cardiol. Méx.*, **47**, 650-657, 1977
- [20] GABRIELLI, M. F. R., OKAMOTO, T., MARCANTONIO, E. and BARBOSA, C. E. M.: Autogenous transplantation of rib cartilage, preserved in glycerol, to the malar process of rats. A histological study, *J. Nihon Univ. Sch. Dent.*, **27**, 87-99, 1986
- [21] FRIEDLANDER, G. E.: Immune responses to osteochondral allografts. Current knowledge and future directions, *Clin. Orthop.*, **174**, 58-68, 1983
- [22] BOYNE, P. J.: Transplantation, implantation and grafts, *Dent. Clin. N. Am.*, **15**, 433-453, 1971

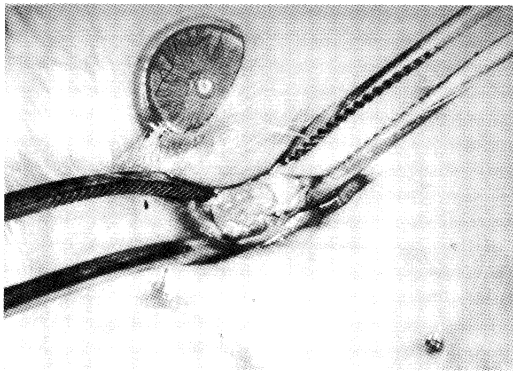


Fig 1 Host bed with the cartilage graft in position

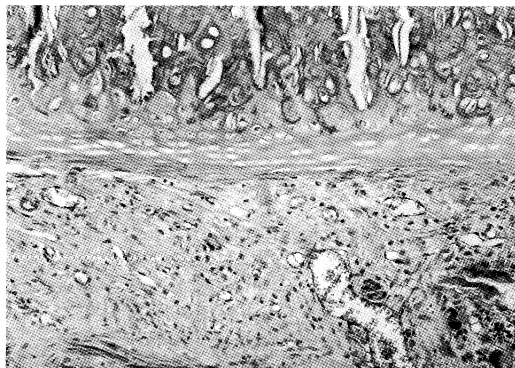


Fig 2 Group I. 10 days. Newly formed connective tissue adjacent to the perichondrium. H. E. $\times 160$



Fig 3 Group II. 10 days. Newly formed connective tissue adjacent to the cartilage without perichondrium. H. E. $\times 160$

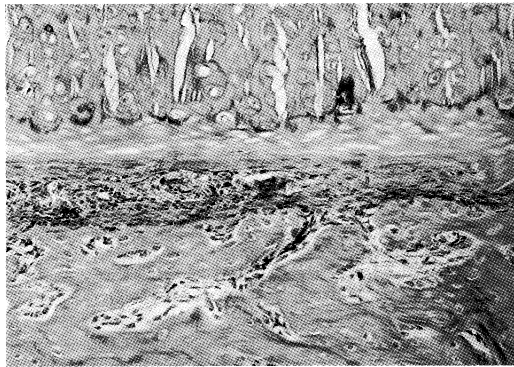


Fig 4 Group I. 20 days. Thin connective tissue lining between the bone and perichondrium. M. T. $\times 160$

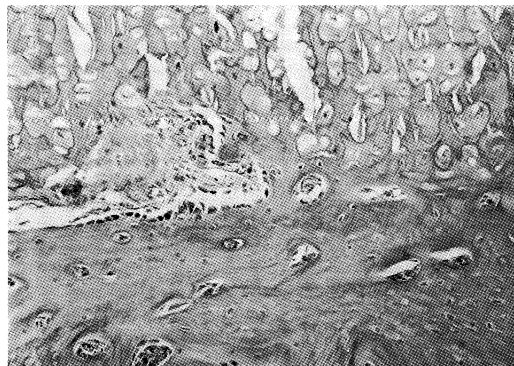


Fig 5 Group II. 20 days. Bone neoformation in close contact with preserved cartilage without perichondrium. H. E. $\times 160$

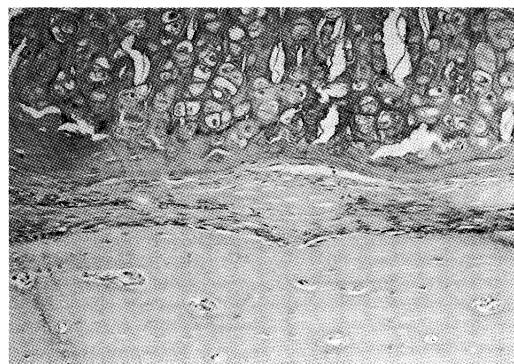


Fig 6 Group I. 30 days. Collagen fibers between perichondrium and newly formed bone. H. E. $\times 160$

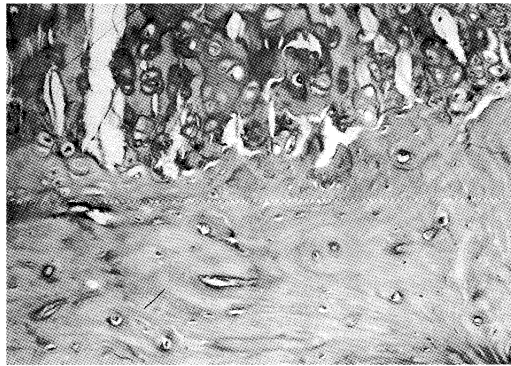


Fig 7 Group II. 30 days. Bone neoformation in direct contact with the preserved cartilage without perichondrium. M.T. $\times 160$

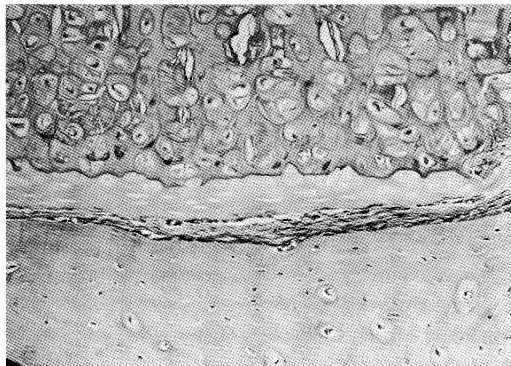


Fig 8 Group II. 60 days. Small space between perichondrium and newly formed bone. H. E. $\times 160$

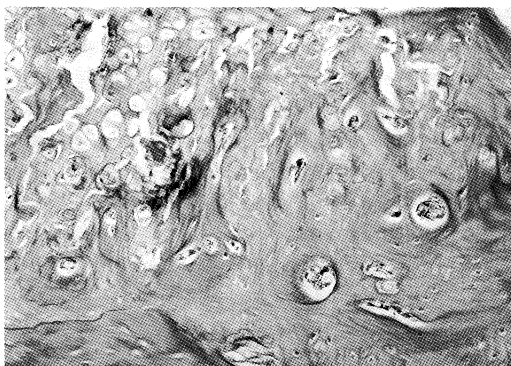


Fig 9 Group II. 60 days. Remaining cartilage associated with newly formed bone. M. T. $\times 160$

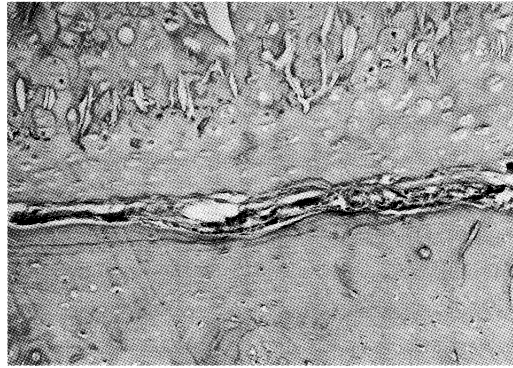


Fig 10 Group I. Connective tissue layer separating the perichondrium and newly formed bone. H. E. $\times 160$



Fig 11 Group II. 120 days. Small amount of remaining cartilage, associated with newly formed bone. M. T. $\times 160$