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Autogenous Transplantation of Apophyseal Cartilage to Osteochondral Defects of Joints

An experimental study in dogs

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P. Benum

INTRODUCTION

The reconstruction of joint surfaces in such conditions as osteochondritis dissecans, defects following unsuccessfully treated joint fractures and dysplastic joints continues to present a problem in orthopaedic surgery. Allotransplantation of osteochondral articular fragments has failed to repair osteochondral defects because of degeneration and disorganization of the cartilage except in some experiments where the transplants have not been thicker than 5 mm (Pap & Krompecher 1961, DePalma et al. 1963, Campbell et al. 1963). It is well known that a metaplastic joint cartilage may be formed when insufficiently developed acetabuli of dysplastic hips are treated with osseous transplants. Such boneshelf operations, however, have often resulted in, or at least have not prevented, the development of osteoarthritis.

Because of the difficulties in the treatment of osteochondral defects of joints, the question has been raised whether autogenous transplants of cartilage of non-articular origin could be of any use in the repair of such defects. Experimental investigations have revealed that costal cartilage may survive transplantation to osteochondral defects of joints (Young 1940, Simons & Danis 1963, Hjertquist & Lemperg 1969, McTavish & Wiley 1969). No report is available, however, concerning clinical application of such transplants except as interposition material (Weglowsky 1907, Peer 1955, Hiertonn 1957).

Previously no attention was directed to the cartilage of the traction epiphyses, or the so-called apophyseal cartilage, as a material that could possibly be used in the repair of defects of joints.

Inasmuch as this type of cartilage is a hyaline one and because it can be easily obtained from the iliac crest without severe damage to the function of the child, it should be of considerable interest to investigate the possibility of restoring defects of joints in children by use of autogenous transplants of such cartilage.

The question whether apophyseal cartilage may be applied in restoring defects of joints does not, however, depend only on whether the cartilage is able to survive the transplantation and heal in with the surrounding cartilage and on its ability to reconstruct the congruency of the joint surface. It also depends on

whether apophyseal cartilage, being a type of cartilage which normally ossifies during adolescence, can be influenced by the mechanical factors of pressure and friction to develop some of the characteristics of joint cartilage of pressure epiphyses, including the quality of being unossifiable.

The importance of the mechanical stimuli in the differentiation of the cells and in the process of ossification of the cartilage in traction- and pressure-epiphyses has not been fully clarified. Some evidence indicates that the mechanical factors of pressure and friction do not play any role in the prevention of ossification of the superficial layers of the cartilage of the pressure epiphyses; the persistence of an unossified cartilage, the future joint cartilage, is possibly governed by genetic factors (McKibbin & Holdsworth 1967). It is, however, well known that the mechanical factors may suppress the ossification of other types of cartilage (Campbell 1931, Krompecher & Goerttler 1938). It therefore seemed reasonable to assume that these factors also might prevent the ossification of the cartilage of traction epiphyses. The validity of this possibility is the main problem of the present investigation.

The first purpose of this study was to investigate by histological methods the changes that occur in apophyseal cartilage after transplantation to osteochondral defects of joints where it is subjected to the same types of mechanical stimuli as the joint cartilage of pressure epiphyses.

Provided that the cartilage survives the transplantation and that the mechanical stimuli from the opposite joint surface prevent the ossification of the cartilage, it might be possible that transplantation of apophyseal cartilage could be applied to restore defects of joints in children. This possibility has been discussed but not explored in the present study.

The following problems have been studied:

1) What changes take place in apophyseal cartilage after autogenous transplantation to ostechondral defects of joints in puppies?
   a) Does the cartilage remain vital after the transplantation?
   b) Do the mechanical stimuli induce changes of the cartilage, making it more like joint cartilage?

c) Does the transplanted cartilage undergo complete ossification or is the process of ossification prevented in the superficial layers?

2) Do the macroscopical and histological findings after autogenous transplantation of apophyseal cartilage to osteochondral defects of joints suggest that transplantation of apophyseal cartilage could be applied as a method of repair of such defects in children?

PREVIOUS INVESTIGATIONS

No record was found of any previous investigation which can throw light upon the presented aspects of apophyseal cartilage. The results of investigations concerning the following subjects are, however, of interest for the present study:

1) The histological structure and the ossification process of apophyses and pressure epiphyses.
2) The viability of cartilage following antogenous transplantation.
3) The influence of mechanical stimuli on chondrogenesis and on ossification of cartilage.

The histological structure and the ossification process of apophyses and pressure epiphyses

Studies of pressure epiphyses (Ham 1965) and apophyses (Ponseti et al. 1968) have revealed that the tissue structure and the process of ossification are in many respects similar in the two types of epiphyses. Both consist of a mass of hyaline cartilage overlying a growth plate. The apophyses, however, are surrounded by a perichondrium, whereas the superficial layers of the pressure epiphyses form the joint cartilage. In the hyaline cartilage overlying the growth plate, vessels are found both in the apophyses and in the pressure epiphyses. In both types of epiphyses an enchondral ossification is found in the growth plate. Furthermore, an epiphyseal ossification centre appears around vessels in the cartilage overlying the growth plate. These so-called secondary centres of ossification appear in post-foetal life in human beings except in the femoral condyles. Generally, the ossicles of the apophyses appear later than those of the pressure epiphyses (Trueta 1968).

The structure details of the growth plate of the apophyses seem to be somewhat different from those of the pressure epiphyses. According to Ponseti et al. (1968), the growth plate in the apophysis of the iliac crest in children contains spindle-shaped cartilage cell clusters rather than columns of swollen cells. The most swollen cells become penetrated by small vessels and an enchondral ossification similar to that found in the growth plate of long bones

takes place in these regions. Calcification and ossification, resembling that at the insertions of the cruciate ligaments of the knee, prevail, however, at the margin of the growth plate, where large bundles of collagen fibres insert into shallow grooves of the underlying metaphyseal bone.

There are many similarities between the two types of epiphyses, concerning the histological details of the secondary centre of ossification.

In the pressure epiphyses the ossification starts around vessels in the centre of the cartilage mass and the following steps of events take place (Trueta 1968): proliferation and subsequent swelling of the cartilage cells, calcification of the matrix between the swollen cells, and finally penetration of small vessels into the lacunae of the swollen cells and bone formation by osteoblasts derived from vascular cells. The ossified area then expands peripherally at the expense of the surrounding zone of swollen maturing cells while a constant division of germinal cells adds to the size of the epiphysis until the end of the skeletal growth is reached. The swollen maturing cells of the epiphysis are, according to Rigal (1962), formed in a layer of proliferating cells close to the expanding bone nucleus, whereas a more superficially located cell layer contributes to the growth of the joint cartilage.

Mankin (1962 a, 1963 a and 1963 b) reported similar findings in his study of pressure epiphyses in immature rabbits. In mature animals, however, he could not find any signs of proliferative activity of the cells in the remaining articular cartilage.

The secondary ossification of apophyseal cartilage has been studied by Ponseti et al. (1968). They found that the secondary ossification of the apophysis of the iliac crest, which appears at the age of eleven to fifteen years in children, in many respects resembles the ossification of the pressure epiphyses. After penetration by vascular buds the ossification centre develops in the middle of the iliac crest where some cells swell and bone is formed by osteoblasts apparently deriving from connective tissue cells surrounding the vessels. Some cartilage cells also appear to change into osteoblasts. Increased cartilage cell division also occurs in the surrounding areas, as seen in the secondary ossification of pressure epiphyses. The secondary ossification centre expands rapidly along the crest but does not fuse to the metaphysis until the late teens. In contrast to the secondary ossification of the pressure

epiphyses, this ossification does not, however, spare the superficial layers of the cartilage.

The viability of cartilage following autogenous transplantation

No report is available concerning the viability of apophyseal cartilage after transplantation. Numerous investigations of transplants of other types of cartilage have been performed, however. Although the results from these investigations have been to some extent conflicting, probably partly because of different techniques employed, there seems to be no doubt that different kinds of cartilage may survive autogenous transplantation into various host tissues provided that the cartilage can be sufficiently nourished by diffusion of nutrients from the surroundings.

Thus, according to investigations reviewed by Ryøppy (1965), epiphyseal cartilage cells may to some extent remain vital and capable of proliferation after implantation into bone or soft tissue in animals. The capability of growth of similar transplants in man, however, has not been demonstrated convincingly, although some investigations seem to reveal that epiphyseal transplants may preserve some potency of growth even in man.

Several studies have demonstrated that articular cartilage may survive autogenous transplantation to osteochondral defects of joints provided that the cartilage is transplanted on an osseous base (for review see Campbell 1963). This finding was also confirmed by Campbell and by DePalma et al. (1963) as well as by Fiala & Herout (1968). Studies concerning the vitality of similar transplants in man have not been reported.

Experiments with animals have revealed that costal cartilage may survive transplantation to osteochondral defects of joints (Young 1940, Simons & Danis 1963, Hjertquist & Lemperg 1969). Weglowski (1907) reported that cartilage having been used as interposition material in an elbow joint was found to be vital five weeks after operation.

Finally it ought to be mentioned that costal cartilage, nasal septal cartilage and ear cartilage may survive implantation in soft tissues, as demonstrated by investigations on grafts employed in plastic surgery in humans and studies on experimentally implanted cartilage in animals (Peer 1955, Gibson 1965). Such cartilage grafts, however, do not seem to possess any capacity of growth in man, in contrast to findings made in some animals (Peer 1955).

The influence of mechanical factors on chondrogenesis and on the process of ossification of cartilage

Mechanical stimuli, according to numerous investigations, influence the process of cartilage formation and the ossification of cartilaginous tissue.

Thus, several in vitro investigations have revealed that mechanical factors are able to influence the cartilage formation from embryonic skeletal tissues. Glücksmann (1939) found that pressure favoured the formation of cartilage from perichondrium and periosteum. Weiss & Amprino (1940) found by experiments on scleral mesenchymal tissue that strong tension tends to suppress the differentiation of chondrogenic tissue into cartilage. Basset & Hermann (1961) concluded from in vitro experiments on tibial cortex tissue of chick embryos that mechanical factors and variation in oxygen concentration can determine the type of connective tissue that differentiates in a culture of cells arising from bone. Compaction and high oxygen concentration favoured development of bone, compaction and low oxygen concentration favoured development of cartilage, whereas stretching and high oxygen concentration stimulated formation of fascia or tendon-like tissue. Hall (1968) found that germinal cells of the membrane bones of the embryonic chick are switched from osteogenesis to adventitious chondrogenesis under the influence of intermittent pressure and tension, and furthermore (1969) that low oxygen concentration initiates such a chondrogenic pathway. The complete differentiation of chondroblasts into chondrocytes, however, was found to be dependent on the mechanical stimuli.

As to the role of mechanical factors during the development of the skeletal system, the general opinion seems to be as cited from Hamilton et al. (1962 p. 408): "Mechanical factors such as pressure or muscular contraction may have a modyfying influence on the details of skeletal shape, but the primary characteristics of general form and size of bones, and the existence and position of joints between bones are due to self-differentiation." This opinion is based upon observations from tissue culture and in vivo experiments under conditions which prevent embryonic movement of bones. It should be noticed in particular that the separation of the articular surfaces during the development of joints and also the formation of joint cartilage are unrelated to external mechanical factors. According to Fell (1956) it seems probable, however, that these events are due to mechanical factors of differential growth. Chondrifying

regions within the future epiphyses of the joint regions of the skeletal blastema enlarge more rapidly than the surrounding tissue through the combined effects of the formation of matrix and cell division. This further causes the cells of the surrounding tissue to become orientated around and incorporated into one or the other of the chondrifying epiphyses until no free cells remain. The joint cartilage formed in this way originates, at least partially, from the undifferentiated mesenchymal tissue of the so-called interzones between the developing bones and from the central parts of the intracapsular perichondrium (Haines 1947).

Neither embryological studies nor tissue culture experiments, however, have presented conclusive evidence that this cartilage is bound to remain unossified whereas the cartilage of the central masses of the epiphyses will be ossified regardless of mechanical factors. Nor has this problem been completely clarified by post-foetal investigations. The findings of McKibbin & Holdsworth (1967) that the most superficial layer of a piece of developing joint cartilage did not ossify after excision and reimplantation upside down in contrast to the originally basal layers, might suggest that the cartilage of an epiphysis is of a dual nature. The findings that bone induction follows transplantation of germinal epiphyseal layer of articular cartilage of young growing animals, whereas thin split-thickness grafts containing only flattened cells of the gliding surface do not (Urist & Adams 1968), might support this suggestion.

These findings do not definitely prove that the cartilage of the central part of pressure epiphyses is bound to be ossified and that the superficial layers do not ossify because of predestined factors. All the experiments might have been performed at a stage when the mechanical factors might have already influenced the differentiation of the cartilage.

The findings of experimental investigations in immature and mature animals (Salter & Field 1960, Trias 1961, Hall 1963, Thaxter et al. 1965) showing no tendency of enchondral ossification of the articular cartilage but degeneration and resorption following prolonged immobilization, however, might also support the view that the mechanical factors do not play any role in the determination of the ossifiability of the cartilage of pressure epiphyses. On the other hand some enchondral ossification has been reported to take place in articular cartilage of human joints following immobilization (Enneking & Horowitz 1972).

During post-foetal life, formation of cartilage is a well-known phenomenon in fracture repair, in repair of osteochondral defects of joints and in different types of interposition arthroplasties. The formation of cartilage during all of these conditions has been attributed to the influence of mechanical stimuli upon mesenchymal cells, which according to Maximow (1926, 1927) may persist in an undifferentiated state in different types of connective tissue even in the mature organism. Thus Krompecher (1935) found during experiments in dogs that pressure caused cartilaginous callus formation from undifferentiated mesenchymal cells, whereas traction favoured formation of fibrous callus. He further found, in cooperation with Goerttler (1938), that the movement of fracture ends would prevent the ossification of cartilaginous callus. This is in agreement with clinical findings concerning non-unions of bones (Campbell 1931). The formation of fibrous or hyaline cartilage from granulation tissue in osteochondral defects of joints as registered by several investigations in both mature and immature animals, has been ascribed to the influence of functional stress as created by early motion and weight-bearing (DePalma et al. 1966). The formation of fibrous or hyaline cartilage in arthroplasties where skin (Kettunen 1958) or vitallium cups (Smith-Petersen 1948, 1952, Gibson & Williams 1951, Jakobsen 1957) have been applied as interposition materials has also been attributed to the factors of pressure and friction.

MATERIALS AND METHODS

Experimental model

The following experiments were performed in puppies. Autogenous transplants from the iliac crest were used. They were removed before a secondary centre of ossification had appeared within the apophysis of the crest.

A: Transplantation of a cylindrical osteochondral fragment, consisting of apophyseal cartilage and adjacent metaphyseal bone, to an osteochondral defect in a weight-bearing part of either the medial or the lateral femoral condyle.

B: Transplantation of a similar fragment to a defect at the medial side of the medial femoral condyle, outside the joint surface. This was done to determine which changes in the former transplants are caused by the transplantation itself and which are caused by the mechanical stimuli.

C: Transplantation of a pure osseous fragment to an osteochondral defect of the weight-bearing part of that femoral condyle which had not been utilized in A. This was done to investigate further the role of the cartilaginous part of the transplants in the repair of the defects.

D: Transplantation of a similar fragment to that applied in A but to a defect as in C and first after devitalization of the chondrous part. This was done to help distinguish changes within necrotic cartilage from changes of cartilage still vital after transplantation.

The transplantations of A and B were combined with those of C in both knees of some animals and combined with those of D in both knees of the rest. The transplants of A were placed in the medial femoral condyle of the left knee and in the lateral femoral condyle of the right, whereas the transplants of C and D were placed in the opposite condyles (Figure 1 A). These combinations were applied to make the mechanical stimuli acting upon the transplants of the groups A, C and D as equal as possible. To find out if the ossification of the transplants is related to the location of the donor site within the iliac crest, the transplants were placed according to a standard system as shown in Figure 1 B.

Animals

Twenty-eight mongrel puppies of both sexes bred in a veterinarian controlled kennel were used in the present study. The age of the animals at the start of the experiments varied between 3 and 4 months. This age level was chosen in accordance with the findings of a radiological pilot study showing that the secondary centre of ossification appears in the iliac crest at an earliest age of 4½-5 months. Four animals were excluded, two because of patellar luxation, one because of infection at the donor site and one because of pregnancy. The final material of 24 dogs consisted of 12 with the combination of transplants A, B and C and 12 with the combination of A, B and D.

Operative procedure

The animals were anaesthetized with Nembutal, 30-40 mg/kg body weight administered i.v.. The operative field was shaved and washed successively with two antiseptic solutions (Benzalkon 1% and iod-spiritus 2%). The operations were performed under aseptic conditions.

Removal of transplants from the iliac crest

The transplants were obtained from the left iliac crest. Following incision of the skin along the crest, the muscular attachments were released and osteochondral transplants were prepared by means of a punch with an internal diameter of 4 mm. The punch was inserted into the apophysis cranially and by rotating and pushing the punch, cylindrical grafts consisting of apophyseal cartilage and adjacent metaphyseal bone were obtained (Figure 2, A and B). The removal of the grafts was completed by cutting through the cartilage and the bone at the lateral side of the punch and by resection of the bone at the base of the punch, which had been inserted 5-10 mm down into the metaphyseal bone. In most cases the apophysis was wide enough to give transplants that did not include any perichondrial tissue medially or laterally. The whole length of the iliac crest had to be used to obtain six osteochondral fragments for preparation of transplants and one for biopsy. During the preparation of the joint defects the transplants were stored in sterile

Ringer's solution for a maximum of 40 minutes. The temperature of the solution was about 20°C.

Further preparation and insertion of transplants

The defects were prepared and the transplants inserted through a medial parapatellar incision, the patella having been luxated laterally.

Tr.pl. A: The defects were prepared as far posteriorly in the joint surface as possible in order to give the transplants a definite weight-bearing function. A punch was used to prepare the defects, which were given a depth of 6-10 mm. The punch had an external diameter like the internal diameter of the device that had been used for removal of the transplants from the iliac crest, that is 4 mm. After preparation of the peripheral limit of the defects, the central part was prepared by means of a drill applied through the lumen of the inserted punch (Figure 3 A).

The height of the cartilaginous part of the transplants varied according to the varying height of the apophysis along the iliac crest and according to the difference of the height of the apophysis in the different puppies. Hence, the depth of the defects had to be individually adjusted to make it possible to restore the congruency of the joint surface. Before the transplants were inserted, their osseous base was trimmed down to a height of 3-4 mm and after the insertion the most superficial part of the cartilage containing the perichondrial layers was excised at the level of the joint cartilage. The final height of the cartilaginous part of the transplants varied between 3 and 6 mm, whereas the height of the surrounding joint cartilage was 1 to 2 mm.

In some transplants a loosening in the growth plate appeared during removal from the iliac crest or during insertion into the defects. These transplants, however, were not eliminated from the study. All the transplants were found to be very firmly attached after insertion. In most cases there was a very close contact between the transplants and the surrounding joint cartilage (Figure 3 B).

Tr.pl. B: The transplants in the non-weight-bearing position were implanted according to the same technique but at the medial side of the medial femoral condyle, just behind the condylar ridge (Figure

3 B). Efforts were made to insert them some distance below the epiphyseal plate and yet keep them out of the region of the weight-bearing transplant.

Tr.pl. C: The pure osseous transplants were prepared by excision of the cartilage from the osteochondral fragments. They were inserted into defects of about the same depth as the osteochondral transplants, giving only a partial filling of the defects (Figure 3 B).

Tr.pl. D: The cartilage was devitalized by heating at 50°C for 30 minutes in sterile Ringer's solution. After insertion of the osseous part of the transplants, which in advance had been separated from the cartilage and not devitalized, the devitalized cartilage was implanted and the surface was trimmed in the same way as that of the transplants A.

After all the transplants had been inserted, the capsule of the joints was closed by interrupted flexafil sutures and the skin by continuous supramid-sutures intracutanously. The same types of sutures were applied at the donor site, for the muscles and the skin respectively.

Postoperative activity

Weight-bearing was allowed from the first postoperative day; all the puppies were then able to walk. From the third or fourth day after the operation most of them started running and two weeks post-operatively, when transferred to the kennel, their function seemed to be almost normal.

Methods of investigations

The animals were sacrificed at the following intervals: two, six and twelve weeks, six, nine and fourteen months. At each period of observation there were two animals which contained the combination of the transplants A, B and C and two with the combination A, B and D, giving eight transplants of type A, eight of type B, four of type C and also four of type D. At twelve weeks and at fourteen months there were only seven transplants of type A avail-

able for histological examination because of technical errors during sectioning.

The maximum length of observation, 14 months, was chosen in accordance with the results of a radiological pilot study revealing that the ossification of the apophysis of the iliac crest is completed at the age of 17 months (14-17 months) in dogs. Radiological examination of the unutilized right iliac crest was nevertheless performed before the animals of the 14 months observation group were sacrificed to ascertain that the ossification really had been completed.

The following examinations were performed in all groups of animals: macroscopical examination of the operated joints, which were photographed, radiological examination of the iliac crest not utilized as donor site, histological and autoradiographic (^{35}S) examinations of the transplants including the adjoining part of the condyles, and of the unutilized iliac crest. The autoradiographic findings will be presented in a separate report.

The dogs were killed by i.v. injection of a lethal dose of Nembutal. Immediately after the sacrifice of the animals, the operated knee joints and the right iliac crest were removed. After the macroscopic examination and photographic recording, the joints were immersed in neutral hexamethylene-tetramin buffered 4 per cent formaldehyde solution for one hour. Then transversally resected 4 mm thick blocks containing the transplant in the weight-bearing position and the adjoining parts of the condyles were removed by means of a saw. In those cases where an ingrowth of joint cartilage was found predominantly over the anterior and posterior part of the transplant, the resection was performed at a slightly oblique angle to the transversal line through the femoral condyles in order to include some parts of this cartilage in the blocks. The transplant at the medial side of the medial femoral condyle including the joint surface anteriorally and the adjoining bone tissue posteriorally was removed by a resection at right-angles to the length axis of the femur (Figure 12 A). In those cases where there could be some doubt concerning the exact position of the transplants the specimens were carefully trimmed before the blocks were resected.

The resected right iliac crest including the adjoining metaphyseal bone was examined radiologically to determine the stage of ossification. Then a block 5 mm thick was removed from its posterior, middle and anterior part by resections at right-angles to the length axis of the crest.

All the resected blocks were then fixed in neutral hexamethylen-tetramin buffered 4 per cent formaldehyde solution for 24 hours, decalcified in 7 per cent solution of nitric acid for 24 hours, thereafter washed in water and dehydrated in ethanol-xylene before embedding in paraffin.

The blocks containing the transplants were cut parallel to the length axis of the transplants stepwise down to the central parts of the transplants; from this region 5-8 μ thick sections were taken for histological and autoradiographic investigations. Sections of the same thickness were cut from the iliac crest specimens at right-angles to the length axis of the iliac crest.

The sections to be studied histologically were stained with haemalun-azophloxine-saffron, Gomori's staining, or with Alcian green (pH 3.6). The haemalun-azophloxine-saffron staining was applied as a standard method to determine the general morphological characteristics of the tissues (Haemalun is a basophilic stain, azophloxine acts as an eosinophilic counterstain and saffron stains the collagens of the tissues yellow). Gomori's staining was used in order to differentiate more clearly fibrous cartilage from hyaline cartilage, the argyrophilic (reticulin) fibrils being stained by this method. Finally, Alcian green was used to assess the acid mucopolysaccharides, which contribute an essential component of the intercellular matrix of cartilage.

The animals were sacrificed over a long range of time and the sections were stained successively following preparation. Hence, the stains had to be renewed several times during the study and the quality of the staining could not be kept absolutely constant.

RESULTS

EXAMINATION OF THE APOPHYSIS OF THE ILIAC CREST

The main purpose of this part of the study has been to clarify the normal morphological features and in particular the stage of ossification of the apophysis at the various times of observations of the transplants. For these reasons the findings will be related to these times of observations, not primarily to the age of the animals. All the animals were, however, between 3 and 4 months of age at the time of operation.

Radiographic findings

The stage of ossification of the cartilage within the apophysis of the experimental animals sacrificed at the various observation periods is shown in Table 1, and typical examples are demonstrated in Figure 4.

The examination revealed no signs of ossification of the apophysis after two weeks' observation. After six weeks of observation a secondary centre of ossification had appeared in the posterior part of the apophysis in only one of the dogs. Later this centre expanded towards the metaphysis, backwards, and further forward along the crest. After twelve weeks' observation, however, the centre was still almost entirely located to the posterior third of the crest. After six months' observation the middle third of the iliac crest was also partially ossified by secondary ossification and after nine months a secondary centre of ossification was seen along all the crest in two of the four dogs. The ossification of the cartilage seemed to be completed at 14 months' observation in the four dogs studied.

Histological findings

Results concerning the presence of a growth plate and a secondary centre of ossification, and whether the ossification had been completed within the various segments of the iliac crest of the experi-

mental animals are given in Table 2. The morphological structure and the changes taking place during the observation period are furthermore illustrated in Figures 5-10.

In all the puppies at the two weeks' observations and in most of the six weeks' observations, the apophysis consisted of hyaline cartilage without any signs of a secondary ossification. A growth plate was seen adjacent to the metaphyseal bone. The cartilage was surrounded by a perichondrial layer, which obviously served as muscular attachment (Figure 5 A). Most of the cells in the main portion of the cartilage overlying the growth plate were distributed randomly. Some pairs of cells ("double cells") occurred, however, containing spindle-shaped nuclei diametrically located within the same capsule (Figure 5 B). Histological signs of a more active cellular proliferation were seen in the upper part of the growth plate, giving rise to flattened cells arranged in columns. The cells of these columns underwent a progressive swelling so that the cells at the bottom of the columns were largely swollen and polygoneal (Figure 6 A). In this area nests of the most swollen cells were penetrated by small vessels, and ossification took place in the matrix between the cartilage cells. No argyrophilic fibrils could be demonstrated in the apophyseal cartilage by Gomoris' staining, except within the matrix septa of the growth plate (Figure 6 B). The matrix of the cartilage was strongly stained with Alcian green, as were most of the cells, except the most swollen ones (Figure 6 C).

During the initial stages of the secondary ossification of any segment of the iliac crest, large cartilage cells, partially arranged in clusters, were found around the ossification centre. Most of these cells contained, in contrast to the most swollen cells of the growth plates, a strongly Alcian-positive cytoplasm and they were surrounded by a strongly Alcian-positive matrix. The ossification of the matrix started where penetration of small vessels into the cell nests could be seen (Figure 7, A, B and C).

During the further process of ossification of any segment of the iliac crest, the cartilage cells did not become swollen to the same degree. Thus, during the final stage the secondary ossification took place without any preceding swelling of the cartilage cells (Figure 8). At this stage neither the cells nor the surrounding matrix showed any greater accumulation of Alcian-postitive substances than the corresponding structures in the rest of the apophyseal cartilage.

The frequency of double cells declined during the time following the first observations; thus double cells were only occasionally seen in the cartilage when the secondary ossification had been well established at the twelve weeks' observations, and at the six months' observations hardly any double cells could be found (Figure 8 B).

During the process of maturation the cell columns, interpreted as signs of proliferation, were also reduced. A regular growth zone containing cell columns of only moderately reduced height was, however, still found in all the segments of the iliac crest at the twelve weeks' observations (Table 2, Figure 9 A). The height of the plate was markedly reduced and the columnar arrangements of the cells had become much irregular, however, at six months' observation (Table 2, Figure 9 B). At nine months' observation most of the apophyseal cartilage had become ossified and growth plates containing regular columns of cartilage cells were practically not observed. In the middle part of the iliac crest, however, there were still some signs of columnar arrangement of the cells in two of the four dogs (Table 2, Figure 9, C and D). Then finally these signs of proliferation also disappeared and the persisting cartilage ossified at the apophyseal side as well as at the metaphyseal side without any preceding swelling of the cells (Table 2, Figure 10, A and B). At the 14 months' observations the apophysis had been completely or practically completely ossified in two of the dogs, whereas only scanty remnants of unossified cartilage could still be found within the iliac crest of the two others (Table 2, Figure 10 C).

Comments

At the radiographic examination a secondary centre was recorded as soon as an ossicle had appeared in some part of the segment. The histological investigations were performed, however, only on sections taken from the middle part og each segment of the iliac crest. Hence there was a slight discrepancy between the histological and radiographic findings concerning the extension of the secondary centre of ossification, owing to the fact that the sections studied histologically were not always taken from the precise area of earliest ossification.

The histological investigation revealed that there was some cartilage left within the iliac crest of some of the dogs at 14

months, in spite of the observation that the ossification had been completed according to the radiographic investigation. It should be emphasized, however, that these remnants of unossified cartilage were very scanty.

EXAMINATION OF THE OPERATED JOINTS

Macroscopical findings

Transplants A

Appearance: At all times of observation up to six months, the surface of most of the osteochondral transplants in positions numbers 2 and 5 appeared to be smooth and shiny, its colour being somewhat whiter than that of the surrounding joint cartilage. Macroscopically it seemed to be cartilaginous. At the three longest times of observation, the surface of some of the transplants was irregular and in some defects the superficial cartilaginous layer seemed to be thinner than the surrounding joint cartilage, the underlying bone being visible through the cartilage.

Level: At two weeks most of the transplants were found to be level with the surrounding joint surface or slightly depressed (Figure 11 A), whereas the transplants appeared to be level with the joint surface or slightly protruding at the later observations (Figure 11 B, C, D and E).

Union: In most defects there were no obvious signs of union between the transplants and the surrounding joint cartilage. From six weeks on, some parts of the periphery of some of the transplants, however, were apparently connected to the joint cartilage by a cartilaginous tissue (Figure 11 B). In joints where the surface of the transplants was depressed, a slight overgrowth of cartilage apparently originating from the joint cartilage was seen peripherally from six weeks on. Only in one defect at 14 months of observation had the major part of the transplant been covered by such cartilage.

Transplants B

The osteochondral transplants which had been placed outside the chondrous joint surface at the medial side of the medial femoral

condyles had from two weeks on been frequently covered superficially by a thin layer of soft tissue apparently originating from the synovial membrane. Up to twelve weeks the cartilage of the superficial part of all the transplants, except one, seemed to be unossified. From six months on, however, some part or the whole of the cartilage in the superficial region of most of the transplants had apparently undergone ossification.

Transplants C

The defects into which only osseous transplants had been implanted appeared to be partially filled with a greyish soft tissue at two weeks. From six weeks and in particular from twelve weeks on, the consistency of this tissue filling the central part of the original defects was considerable firmer. From six weeks on, the area of the defects had been reduced to a varying extent by ingrowth of joint cartilage peripherally, in particular anteriorally and posteriorally. A central circular or transversal linear defect was always found, however, and the joint surface corresponding to the original defect and some part of the surrounding joint surface was, as a rule, found to be slightly depressed (Figure 11, A and B). The minimum width of the smallest remaining defects was less than 1 mm.

Transplants D

In the defects into which bone fragments and devitalized cartilage had been implanted, a reduction of the area of the defects had taken place peripherally to approximately the same extent and apparently in the same manner as in the defects mentioned above (Figure 11, C and D).

During removal of the operated joints all the transplants which had been placed within the femoral joint surface were found in the anterior part of, but well within, the surface of the tibio-femoral joint. The menisci, being very broad in dogs, constituted a major part of the opposite joint surface.

None of the joints appeared to have been infected and there were no signs of osteoarthritis, except in two joints at nine months and in one joint at 14 months, in which there was a marked osteoarthritis of the patello-femoral part of the joint.

A slightly damaged cartilage was found by inspection of the tibial joint surface corresponding to the surface articulating against the osteochondral transplants, in particular from six months on, in joints where the transplants protruded significantly, but not when the transplants did not protrude. The cartilage of the joint surfaces opposing the pure osseous or the devitalized cartilage implants was generally found to be somewhat more damaged.

H i s t o l o g i c a l f i n d i n g s

Transplants A

Various changes took place following the transplantation of the osteochondral fragments from the iliac crest to the osteochondral defects of the femoral condyles. In order to elucidate the problems raised in this investigation (p. 3) it was found to be of particular interest to study histologically

1) The signs of vitality of the transplanted cartilage.
2) The cellular changes and the changes taking place within the matrix of cartilage that had survived the transplantation.
3) The presence of connective tissue and the transformation of such tissue to cartilage.
4) The fate of necrotized cartilage.
5) The ossification of the transplanted cartilage.
6) The changes of the osseous part of the transplants.
7) The changes of the surrounding joint cartilage.
8) The process of union between the transplants and the surrounding tissue.
9) The surface level of the transplants.

To simplify the presentation of the findings, the cartilage of the transplants has been divided into three different zones due to the different fate of the cartilage of the various parts of the transplants (Figure 12 B).

zone a: The superficial half of the cartilage located above the base level of the surrounding joint cartilage.
zone b: The area between zone a and the base level of the surrounding joint cartilage.
zone c: The area between zone b and the original location of the growth plate.

The vitality of the transplanted cartilage

Criteria: Cells were considered to be vital when they contained haemalun-stained nuclei of normal size and stainability and when normal appearance of the cytoplasm was demonstrated by the haemalun-azophloxine-saffron staining (Figure 13 A). Pycnotic nuclei, sometimes located peripherally in the cells, and loss of normal structures within the cytoplasm were taken as signs of necrosis of the cells (Figure 13 B).

Lack of Alcian-stainable cytoplasm was taken as a confirmative sign of necrosis if the cells contained a peripherally located pycnotic nucleus (Figure 14 C).

Findings: Results are summarized in Table 3. At the shortest time of observation, two weeks, necrotic areas were always found in the basal-central regions of the cartilage, affecting some part of the growth plate and the overlying zone c. The extent of the necrosis varied widely between the various transplants. In two of them the growth plate had necrotized completely whereas only the middle half of this zone had been affected in one transplant. In the five other transplants more than two thirds of the growth plate had necrotized. The middle zone, zone c, also appeared to be necrotic to a varying extent. In four transplants there was hardly any necrosis of this zone, in the others a more extensive necrosis involving the central area of the basal part but comparatively less of the more superficial part of the zone was found. In all transplants where some parts of zone c had necrotized, the necrotic area became gradually narrower towards the joint surface. It never extended into the superficial zones a or b (Figure 14). In addition to the necrotic areas described above, a narrow peripheral necrotic zone involving two to four rows of cells occurred in every transplant (Figure 26 A).

The maximal and minimal extensions of the necrotic areas are shown schematically in Figure 16.

At the later observations the demonstrable necrotic regions encompassed comparatively smaller parts of the transplanted cartilage than at two weeks. It is particularly noteworthy that there were no signs of necrosis of the most superficial zones a and b, except in one transplant at 14 months.

Comments

At two weeks the determination of the original size of the cartilaginous part of the transplants offered no particular difficulties. The peripheral borders were well defined since no ossification had taken place peripherally (p. 29) and since the necrotized part of the growth plate was still to be seen (Table 3, Figure 15). At the later observations the size of the originally cartilaginous part of the transplants was less clearly defined. Some parts of the cartilage which had survived the transplantation had undergone ossification (p. 29) and furthermore, necrotized cartilage had to some extent been replaced by bony tissue (p. 28). Hence, the areas of necrotic cartilage present at the later observations (Table 3) give no exact information about the original extension of the necrosis of the cartilage in the examined transplants. It is worth while noticing, however, that there was generally no indication of further necrotizing of the transplanted cartilage after two weeks of observation.

Cellular changes and changes within the matrix of cartilage that had survived the transplantation

Cellular changes

Various changes had taken place among the vital cartilage cells. Only those which are supposed to throw some light upon the effect of the transplantation and upon the influence of the joint function on the apophyseal cartilage will be presented here. These are: changes in the proliferative activity, changes in the size of the cells, changes in the orientation of the cells.

Changes in the proliferative activity

The following findings have been taken as indications of cellular proliferation: 1) Presence of a vital growth plate containing columns of cells of which the most superficial cells are flattened and arranged vertically to the length axis of the columns (Figures 15 C, 18 B). 2) Presence of double cells or cluster cells (Figures 25, A and B, 26 A).

No attempts were made to quantify the proliferative activity, except that the width of the growth plate was measured.

Presence of a vital growth plate

Results are summarized in Table 4. At the shortest time of observation, two weeks, it is seen that only scanty remnants of growth plates had survived the transplantation (Figures 14 A, 15 C). Thus the width of the vital part of the growth plates did not exceed one third of the total width of the transplant, except in one case. At six weeks, however, a vital growth plate was seen through all the width of the transplant in six of the eight transplants. In two of the transplants this growth plate was located above the remnants of a necrotized growth plate (Figure 17, B and C). At twelve weeks' observation a complete growth plate occurred more rarely and the proliferative activity within the remaining growth plates seemed to be very low since the cell columns were short and irregular (Figure 18 B). From six months on no growth plates or fragments of such plates could be seen.

Comments

The presence of cells arranged in columns only represents the result of the proliferative activity in the basal region of the presisting cartilage of the transplants. The findings of a vital growth plate superficial to remnants of a necrotized growth plate in some transplants at six weeks, however, should clearly indicate that regeneration of the growth plate had taken place after the transplanted growth plate had partially necrotized. This could also be suggested from the findings that the growth plates were much broader six weeks than two weeks after the transplantations. It should be noted, however, that growth plates were present far longer within non-transplanted than within transplanted apophyseal cartilage (compare Table 2 to Table 4).

Presence of double cells and cluster cells

Two, six and twelve weeks after transplantation double cells were occasionally seen. Such cells were found more frequently in the peripheral regions adjacent to the narrow necrotic brim (p. 23) than more centrally. In the same regions some cell clusters were also observed (Figures 25, A and B, 26 A). At the later observations there were practically no double cells or cluster cells to be seen.

Comments

The presence of two or more cells located within the same capsule, or lying very closely together, should indicate that proliferation has taken place (Carlson 1957, Hulth et al. 1970). The findings of such cells within the transplanted cartilage do not, however, prove that cell division really has occurred a short time before the sacrifice of the animal. The fact that an increased occurrence was found in the peripheral regions of the transplants, nevertheless, indicates that at least some of these cells had arisen after the cartilage had been transplanted. Besides, cluster cells were not observed within apophyseal cartilage at the time of transplantation.

Changes in the size of the cells

After six weeks there appeared to be some difference in some of the transplants concerning the size of the cells of the various zones. Thus the cells of zone b and/or zone c were significantly larger than the cells of zone a in some transplants. The cells of the latter zone were never found to be significantly larger than the cells of the joint cartilage, not even at the later observations (Figures 22, B and C, 23, B and C).

The occurrence of differentiation concerning the size of the cells of the various zones is shown in Table 5. An example of such differentiation is shown in Figure 19, A and C. As seen from Table 5 the phenomenon of increased size of the cells of zone c was found inconsistently from six weeks on, except at 14 months, when it was present in all the transplants which still contained cartilage of apophyseal origin in this zone. At this observation time even the cells of zone b appeared to be slightly swollen in two of the three transplants which still contained unossified cartilage of apophyseal origin in this zone (Table 8). The slightly swollen cells all contained Alcian-positive cytoplasm (Figure 19 D).

Comments

In most transplants where swollen cells were seen in zone c and/or zone b the cells were only slightly swollen. Compared to the most swollen cells found during the initial stages of the secondary ossi-

fication within the iliac crest (p. 18), their size was definitely smaller. The possible relationship between the swelling of the cells and the ossifiability of the matrix will be considered later (p. 55).

The orientation of the cartilage cells

Results are summarized in Table 6. From six weeks on the most superficial cells of zone a tended in some transplants to be flattened and to orient themselves with their long axis parallel to the joint surface. An example of this phenomenon is shown in Figure 20 B. This tendency was not found consistently, however.

Comments

In those cases where the most superficial cells appeared to be flattened and oriented as mentioned above, this contributed to make the transplanted cartilage more like joint cartilage, the latter being covered superficially with a layer of "lining cells" (Figure 21 C).

Matrix

The matrix of the vital regions of the transplanted cartilage was Alcián-positive at all periods of observations (Figure 19, B and D). No fibrous elements could be detected within the transplanted cartilage by Gomori's staining, except in the basal part of the matrix septa between the columns of swollen cells in those transplants that still contained a growth plate.

Comments

The stainability of the matrix with Alcian green indicates that acid mucopolysaccharides were still present within the cartilage (Pearse 1960, Földes et al. 1970). Since the staining method was not exactly standardized for quantitative analysis, no comparison can be made between the amounts of these substances present at the various observation periods. The Gomori's staining confirmed that the apo-

physeal cartilage preserved its hyaline character following transplantation.

Presence of vessels and connective tissue/fibrous cartilage

Connective tissue was found to cover the surface of the transplant in only one defect at two weeks. Fibrocartilage was seen in the superficial part of one defect at six months and in another two at 14 months (Table 3).

There were no signs of ingrowth of vessels or connective tissue into the cartilage of zone a or b. In the vital regions of zone c, however, vascular connective tissue buds were occasionally seen from six weeks on.

Comments

It is most unlikely that the fibrous cartilage seen in the defects mentioned above represents cartilage formed from transplanted cartilage, since no signs of transformation of hyaline cartilage to fibrous cartilage were seen during the previous observation periods. The cartilage was possibly formed by metaplasia from connective tissue that had grown over the surface of depressed transplants (compare p. 43).

Fate of necrotized cartilage

The presence of connective tissue, connective tissue undergoing cartilaginous metaplasia, and slightly fibrous cartilage within areas of necrotic cartilage and the presence of ossification of such cartilage were observed, and the results are summarized in Table 7.

In the necrotic regions of the transplanted cartilage a vascular connective tissue was seen in most of the transplants at two weeks. This tissue contained elongated fusiformed cells of the same type as those seen in Figures 26 A, 34 B and 38 B. At six weeks metaplastic formation of cartilage from connective tissue cells was seen within such an area in one transplant and this newly formed cartilage, which replaced the necrotic one, underwent ossification (Figure 24 A). At the same observation period, and at the following

ones up to six months, islands of only slightly fibrous cartilage, some of them undergoing ossification, were found within necrotic areas of some transplants (Figure 24, B and C).

Comments

Although metaplastic formation of cartilage from connective tissue was seen in only one transplant, it seems reasonable to assume that formation of new cartilage and furthermore ossification of such cartilage have been responsible for the reduction of the observable necrotic areas registered from six weeks on (Table 3). The morphological study gave no other clue to the problem how the necrotized cartilage was removed. Furthermore, this was also the mechanism which was found to be responsible for the replacement of cartilage devitalized by heat (p. 45).

The ossification of the transplanted cartilage

The ossification of cartilage that survived the transplantation was found to take place in two different ways:

1) Ossification in the growth plates in the basal part of those transplants that contained such a zone (Figures 17 C and 18 B).
2) Ossification from the basal side of the transplants, without the existence of a growth plate, or ossification of the cartilage of the zones b and c from the periphery. This type of ossification took place without any preceding marked swelling of the cartilage cells (Figure 20 C) and was mainly seen from twelve weeks on.

The extent of the ossification of the various zones is shown in Table 8. The ossification was particularly intensive in the growth plates at six weeks observation. At twelve weeks this ossification has subsided and from six months on it no longer contributed to the ossification of the cartilage since growth plates were no longer seen.

The extent of the ossification of the zones b and c was found to be increased with increasing length of the observation period. Thus the major part of zone c had undergone ossification in most transplants at 14 months. Additionally the cartilage of zone b had become completely ossified in one transplant at nine months and in

two transplants at 14 months, whereas there were no signs of ossification within the most superficial part - zone a - of any transplant.

In those transplants which had become completely ossified to the base of zone a at nine and 14 months, the basal zone of the remaining cartilage resembled the corresponding zone of the surrounding articular cartilage. It contained pale cells embedded in matrix deeply stained by the haemalun-azophloxine-saffron staining technique (Figures 22 and 23). At nine months a similar zone was registered in one transplant where the ossification had reached the base of zone b (Figure 21). However, the full development of such a zone, well demarcated by a distinct cementing line, was not seen until the ossification had reached the base of zone a. Within or beyond this zone there were then no signs of further ossification (Figures 22, A and B, 23, A and B).

A comparison between the extent of the ossification of the cartilage of the transplants taken from the posterior and anterior part of the iliac crest did not show any correlation between the degree of ossification and the location of the donor site within the iliac crest.

The trabecular structure of the bone tissue formed by ossification of the transplanted cartilage was varying, even at the long-term observation of nine and 14 months. In some joints there was a more compact bone structure in the area underlying the persisting transplanted cartilage than below the surrounding joint cartilage (Figures 22 A and 23 A). In other joints, however, the structure of this tissue was quite similar to that of the adjacent condyle area (Figure 21 A).

Comments

As mentioned previously (p. 24) the original size of the cartilaginous part of the transplants could not be exactly determined at the later observations. Hence, the rate of ossification could not be measured accurately. The values given in Table 8, however, should represent the approximate degree of ossification of the various zones. It should be noted in particular that the ossification never extended into the most superficial zone a and furthermore that a zone resembling the calcified zone of mature articular cartilage had developed in the basal part of the persisting carti-

lage in those transplants where the ossification had passed the base level of the surrounding joint cartilage.

Three of the seven transplants available for histological examination at 14 months had to be eliminated when the influence of the mechanical factors upon the process of ossification of apophyseal cartilage was evaluated. This was done because the superficial part of the defect had been filled with fibrous cartilage in one case and with cartilage predominantly of joint cartilage origin in another, and because there appeared to be extensive necrotic changes and fragmentation of the superficial part of one transplant (Table 8).

The osseous part of the transplants

The vitality of the transplanted bone tissue was evaluated according to the following morphological criteria: Normal size and stainability of the cell nuclei indicated vitality, whereas pycnotic cell nuclei or empty bone lacunae indicated cell necrosis. (These criteria were also applied to the pure osseous transplants and they are illustrated in Figure 34 D).

At two weeks, necrotic osteocytes were seen within the central parts of the bone trabeculae. The more superficially located osteocytes and the lining osteoblasts appeared to be vital. The marrow spaces of the transplanted bone tissue contained far fewer mature blood cells and fat cells than those of the surrounding bone tissue (Figure 15, A and B).

At the later observations, however, necrotic osteocytes were only occasionally seen. Furthermore, the marrow spaces appeared to be filled with blood cells and fat cells. In most cases no marked difference could be registered between the structure of the bone trabeculae in the transplanted bone tissue and in the surrounding femoral condyle (Figure 21 A).

Comments

It was not within the scope of this investigation to study the fate of the transplanted bone tissue in detail. It should be noted, however, that there were no signs of resorption of the transplanted bone which could favour break-down of the overlying cartilage.

Changes in the surrounding joint cartilage

A marginal necrotic zone comprising 2-5 rows of cells was found at all times of observation, the same criterion of cell necrosis being applied as during the evaluation of the vitality of the transplanted cartilage (p. 23). In those specimens where the surface of the transplants was found to be slightly depressed, a small lip of cartilage projected into the defect and covered the transplant (Figure 17 A). Within this cartilage cluster cells were seen peripheral to the marginal necrotic zone (Figure 25 A). In one particular transplant at 14 months, where no apophyseal cartilage could be seen in the superficial part of the defect, the ossified deeper part of the transplanted cartilage was found to be almost completely covered with cartilage of the same morphological appearance as the surrounding joint cartilage (Table 3).

Comments

The registered findings strongly indicate that the joint cartilage possessed some ability to grow over the transplanted apophyseal cartilage if the surface of the latter was situated at a lower level.

The process of union between the transplants and the surrounding tissue

In specimens where a narrow cleft was found between the transplant and the surrounding joint cartilage, no signs of union between the transplanted cartilage and the joint cartilage could be found (Figures 15 A, 17, A and B, 18 A, 20 A, 22 A, 23 A, 25 A). If this cleft appeared to be wider than about 0.3-0.4 mm an ingrowth of connective tissue, containing elongated fusiformed cells, was seen at two weeks (Figure 25 B) and at the later observations fibrous or hyaline-like cartilage connected the transplanted cartilage to the surrounding joint cartilage (Figures 25 C, 17 A, 21 A, 23 A).

In the cleft between the transplanted cartilage and the surrounding bone tissue a similar connective tissue as mentioned above was also found at two weeks. This tissue had partly grown into the peripheral necrotic zone of the transplanted cartilage (Figure 26 A) and at six weeks a slightly fibrous cartilage was

also seen within this cleft (Figure 26 B). At the later observations, the transplants appeared to be united to the surrounding bone tissue by such cartilage or by bone tissue formed by ossification of the transplanted cartilage (Figures 21 A, 22 A, 23 A).

Comments

Transformation of connective tissue cells to cartilage cells was not directly observed. The fact that fibrous or even hyaline-like cartilage was seen in the clefts between the transplants and the surrounding tissue from six weeks on whereas only relatively undifferentiated connective tissue was observed at two weeks, however, indicates that such metaplastic formation of cartilage had taken place.

The surface level of the transplants

The microscopical examination of the sections, which had been taken nearly transversally through the central regions of the transplants, revealed that the surface level varied within each animal and within each observation group. A marked change in the surface level appeared to take place, however, during the time of observation. At two weeks a slight depression of the transplants was most commonly found. Later the transplants were on a level with the surrounding joint cartilage or slightly elevated: the maximal protrusion found was 1.4 mm (Figure 18 A). It is worth noting that the transplants were on a level with the surrounding joint surface in those cases where the transplanted cartilage had fused with the joint cartilage (Figures 18 A, 21 A, 23 A).

Comments

It seems reasonable to assume that the slight tendency of some of the transplants to protrude might be related to the presence of a vital growth plate (Table 4) during some part of the period following transplantation. Union of the transplanted cartilage to the articular cartilage seemed to prevent disproportinate growth of the cartilage.

Transplants B

The morphological changes taking place within these transplants will be consecutively compared to those seen within the transplants to the defects of the joint surfaces. The possible reasons for the different fate of the cartilage following transplantation to the two different locations, will, however, be dealt with during the general d60cussion (p. 46).

The cartilage of these transplants has also been divided into three zones. The height of zones a and b was considered to be the same as that of the corresponding zones of the transplant to the joint surface of the same femoral condyle.

The criteria applied during the interpretations of the findings were the same as for the latter transplants.

The vitality of the transplanted cartilage

The results are summarized in Table 9. At the shortest time of observation, two weeks, the major part of the chondrous portions of the transplants appeared to be vital. Necrotic regions were found, however. In four transplants the growth plate had necrotized completely, in the other four only the central part of the growth plate had became necrotic (Figure 27, B and C). The necrotic regions also included the central part of the overlying cartilage of zone c in six transplants. They generally became narrower towards the surface. The superficial zones a and b were vital in all the transplants except that a narrow peripheral necrotic brim involving two to four cells was seen in all the transplants.

At the later observations the observable necrotic areas were generally far smaller than at the earliest observation. It should be noted, however, that small areas of necrotic cartilage were found within the superficial zones a and b of some transplants after 12 weeks of observation.

Comments

It seems reasonable to assume that the average extent of necrosis of the transplants had initially been similar within the transplants of the various observation periods. Ingrowth of connective tissue,

metaplastic formation of cartilage and ossification of such cartilage were most probably the mechanisms responsible for the reduction of the size of the observable necrotic areas from six weeks on (p. 38).

The size and distribution of the necrotic areas (Table 9) were very similar to those seen within the transplants to the defects of the joint surfaces (Table 3), except that small necrotic areas were also seen occasionally in zones a and b in the transplants B at the later observations.

Cellular changes and changes within matrix of cartilage that had survived the transplantation

Presence of a vital growth plate

Results are summarized in Table 10.

A vital growth plate or great parts of such a plate were most commonly seen at six weeks. Both at this observation period, and at two weeks, remnants of necrotized parts of the transplanted plate were observed below vital growth plates (Figure 27, A and B).

Both twelve weeks and at six months following transplantation a vital growth plate was seen through some parts of the transplant in only two out of eight transplants (Figure 27 C). Later there were no signs of proliferating growth plates.

Comments

As in the transplants to the defects of the joint surfaces, the presence of more complete growth plates at six weeks than at two weeks, and furthermore the presence of necrotized parts of growth plates located below vital growth plates, indicated that regeneration of the growth plates had taken place. Since the central columns of the necrotized cells in some transplants converged upwards, as if they were compressed from an increasing mass of cells peripherally (Figure 27 B), the regeneration of the growth plates seemed to take place at least partially from the peripheral surviving parts of the growth plates.

There did not seem to be any significant difference between the occurrence of vital growth plates in the two types of transplants

(compare Table 4 to Table 10). In the cartilage transplanted to defects outside the joint surface, the proliferation of the growth plates also ceased earlier than within non-transplanted cartilage (Compare Table 1o and Table 2).

Presence of double cells and cluster cells

Two weeks after transplantation double cells were seen more frequently in the cartilage adjacent to the narrow peripheral necrotic brim than in the central regions. Also, cluster cells were occasionally seen in the same areas. At the later observations swollen cluster cells were seen in some transplants in the ossification areas (Figure 28 C), whereas double cells were not observed in these areas more frequently than in the rest of the cartilage.

Comments

The findings indicated that proliferation gave rise to double cells and cluster cells first of all during the first two weeks after transplantation. Compared to the cartilage transplanted to the defects within the surfaces of the joints, the findings concerning this type of proliferation did not seem to be significantly different. It should be noted, however, that no exact quantitative analysis has been performed.

Changes in the size of the cells

No particular difference was found at any observation period concerning the susceptibility of the cells of the different zones a, b and c to become swollen. Some markedly swollen cells could be found in all these zones in some transplants at twelve weeks. These cells and the surrounding matrix, were strongly stained with Alcian green (Figure 29, C and D).

Comments

Compared to the findings in the cartilage transplanted to defects within the surface of the joints, the most striking difference was

that all the cells of the most superficial zone of the latter remained small through all the observation periods. Markedly swollen cartilage cells, as seen during the initial stage of ossification in the iliac crest (p. 18), were apparently more frequently found in the cartilage transplanted to defects outside the joint surface, at twelve weeks' observation, than in the cartilage transplanted to the defects of the joint surfaces. Even in the former transplants, however, markedly swollen cells were only found inconsistently.

The orientation of the cartilage cells

The cells of all the zones, except those of the growth plates, were randomly oriented during all periods of observation.

Comments

These findings differed from those concerning the orientation of the cells of the transplants to the defects of the joint surfaces, in which the most superficial cells of some transplants tended to orient themselves parallel to the surface of the transplants (p. 27).

Matrix

The findings were similar to those of the transplants to the joint surfaces, except that a strongly Alcian-stained matrix was found around the swollen cells mentioned above (Figure 29 D).

Presence of vessels and connective tissue

At all periods of observation vascular connective tissue buds were occasionally seen in all the zones of the cartilage. In some transplants the most superficial zone had apparently been invaded by connective tissue covering the surface of the transplants (Figure 28 A).

In this group of transplants the connective tissue cells were also relatively long and fusiformed at two weeks, whereas they were definitely rounder at the later observations (Figure 32). No signs

of ossification were seen around such connective tissue buds. There were no signs of formation of fibrous cartilage within vital apophyseal cartilage.

Comments

The most significant difference concerning the occurrence of vascular connective tissue within the cartilage transplanted to the two different locations was that only the transplants outside the joint surface contained such tissue in their most superficial zone. Furthermore, connective tissue covering the surface of the transplants was not seen among the latter transplants, with only one exception (Table 3).

Fate of necrotized cartilage

Connective tissue, slightly fibrous cartilage and ossification of the latter were observed within the necrotic cartilage of the transplants. The results are summarized in Table 11.

Vessels and connective tissue were seen in the necrotic regions two weeks after transplantation and occasionally later (Figure 33 A). Slightly fibrous cartilage was seen within areas of necrotized cartilage occasionally from six weeks on (Figure 33 B). Ossification of such cartilage, however, was observed in only one case at six weeks (Figure 33 C). At the latest observations, 14 months, no necrotic cartilage could be observed in four of the transplants and in none of the transplants could fibrous cartilage be observed.

Comments

Although metaplastic transformation of connective tissue to cartilage was not observed in any case, it seems reasonable to assume that the islets of slightly fibrous cartilage seen within the necrotic areas had been formed by such metaplasia, as demonstrated in the transplants to the defects of the joint surfaces (p. 28) and in the devitalized cartilage controls (p. 45). Furthermore, replacement of such cartilage by bone seems to be the most probable mechanism by which the necrotic cartilage areas also finally disappeared in this group of transplants.

The ossification of the transplanted cartilage

The cartilage that survived the transplantation was replaced by bone in two different ways:

1) Ossification in the growth plates in the basal part of those transplants that contained such a zone (Figure 27 C).
2) Ossification from the basal side of the transplants without the existence of a growth plate or ossification from the periphery (Figures 28, A and C, 29, A and B). Such ossification was seen from twelve weeks on. Occasionally double cells, cluster cells and swollen cells were seen in the ossification area (Figures 28 C, 29 C).

The extent of the ossification is shown in Table 12. Ossification was seen in the growth plates in some transplants at all observation periods up to six months. Such ossification was most extensive and most frequently seen at six weeks. The table further shows that the cartilage of zones a, b and c was generally replaced by bone to an increasing extent from twelve weeks on. Thus some parts of the most superficial zone, zone a, appeared to be ossified in all the transplants, except one, at 14 months. Two of the eight transplants had then become completely ossified (Figure 31 A) whereas another two contained only insignificant remnants of cartilage within zones a and b.

Transplants that protruded tended to contain some unossified cartilage (Figure 31 B).

Four of the transplants of the six weeks' observations had been inserted with one side through the epiphyseal plate. There were, however, no signs of ossification of the transplanted cartilage near the epiphyseal plate (Figure 28, A and B).

Transplants taken from the posterior part of the iliac crest did not ossify earlier or more extensively than those taken from the anterior region.

Comments

The way in which the ossification took place did not differ significantly from that seen within the cartilage transplanted to the defects of the joint surfaces, except that the cartilage cells of the ossifying areas underwent more distinct swelling in some of

the transplants.

The extent of ossification was, however, far greater than in the cartilage transplanted to the joint surfaces (compare Tables 12 and 8). Furthermore, it was of particular interest to note that, at the later observations, the ossification also included the most superficial zone a, in contrast to what was seen in the transplants to the joint surfaces.

The osseous part of the transplants

The findings did not differ from those of the transplants to the joint surfaces.

The process of union between the transplants and the surrounding tissue

At all times of observations most transplants were found to be overgrown by fibrous connective tissue originating from the periosteum or the synovial membrane. The thickness of this connective tissue varied widely (Figures 29 A, 30 B). There were no signs of ossification of the cartilage around vessels that had penetrated into the transplants from this connective tissue layer. The deeper part of the transplants was connected to the bone bed by means of connective tissue at two weeks, fibrous cartilage at six weeks and of bone tissue at the later observations.

Comments

Except in the most superficial part of the defect, the process of union between the cartilage and the bone bed seemed to be exactly the same as that seen in the defect of the joint surfaces (p. 32).

Transplants C and D

In order to clarify further whether the cartilage observed at the late observations of transplants A really originated from transplanted apophyseal cartilage the quality of the changes taking place from time to time within the controls C and D was studied.

Transplants C

Changes within granulation tissue

At two weeks the defects appeared to be partially filled with granulation tissue which apparently originated from the marrow spaces of the surrounding bone tissue. This tissue contained relatively few fibres but was rich in oval or spindle-shaped cells. In the basal part of the defects there were plenty of small vessels. Superficially the cells and the fibres appeared to be oriented parallel to the joint surface. The intercellular substance of the tissue in this part of the defects stained faintly with Alcian green (Figure 34, A, B and C).

At six weeks the defects were found to be almost completely filled with connective tissue relatively rich in cells but poor in fibres. The cells appeared to be more oval or round and there were fewer spindle-shaped cells than at two weeks (Figure 35 A). In two of the defects, which had been almost completely covered superficially with a cartilage that was continuous with the surrounding joint cartilage (compare to the changes of the latter, p. 42), a fibrous cartilage was found centrally and basally (Figure 36, A and B). The matrix of this cartilage was strongly Alcian-positive. Deep in one of these defects, large swollen cartilage cells and enchondral ossification were seen (Figure 35 B). There also appeared to be some ossification within connective tissue that did not contain any cartilage cells.

At twelve weeks the cells of this type of cartilage were generally richer in cytoplasm than at the previous observations.

From six months on no further changes in this cartilage seemed to take place, except that the cartilage in the basal part of the defects became ossified. Thus the persisting cartilage of the central parts of the defects appeared still to be fibrous in most defects up to 14 months; only in some defects a more hyaline-like cartilage was seen. In three out of the four defects at 14 months the cartilage had become ossified up to a level slightly superficial to the basal level of the surrounding joint cartilage. In the basal part of the persisting cartilage, a zone resembling the calcified zone of the surrounding joint cartilage was seen. Neither within this zone nor within the corresponding zone of the joint cartilage were there any signs of further ossification and there were no signs of vascularization of the overlying cartilage (Figure 37).

Changes within the surrounding joint cartilage

At two weeks the superficial half of the joint cartilage projected slightly into the defects and there were more double cells in the layers underlying the lining cells, i.e. the intermediate stratum and the radiate stratum, adjacent to the defects than more peripherally. In the same regions there were also some tendency towards cluster cell formation (Figure 34 B).

From six weeks on, the fibrocartilage of the defects had been covered peripherally by cartilage that morphologically appeared to resemble hyaline joint cartilage. This cartilage was continuous with the joint cartilage surrounding the original defect but was more cellular than this, and contained more cluster cells than the cartilage that was seen at the margins of the defects at two weeks. Centrally this cartilage was demarcated by a necrotic zone encompassing 3-5 rows of cells just like the cartilage surrounding the defects at two weeks. A very clear demarcation from the underlying fibrocartilage was also seen (Figure 36). This cartilage contributed to a varying extent to the healing of the superficial part of the defects. At six weeks there persisted only a narrow transversal cleft of the original defects in two cases (Figure 36 A), whereas the areas of the two other defects had been only slightly reduced peripherally. The degree of reduction of the superficial part of the defects by overgrowth of such cartilage appeared to be approximately the same as at six weeks at the later observations, or slightly increased. In most of the joints, however, there was slight depression of the joint surface corresponding to the original defects.

The osseous part of the transplants

At two weeks many of the osteocytes appeared to be necrotic; however, an extensive osteoblastic activity was seen (Figure 34 D).

At six weeks one of the transplants had been resorbed. At the later observations the findings did not differ significantly from those of the osseous part of the osteochondral transplants A.

Comments

The findings of spindle-shaped connective tissue cells in the defects at two weeks, and more oval or rounded cells at six and twelve weeks indicate that the fibrous or even hyaline-like cartilage observed in the central parts of the defects at the later observations had been created by transformation of relatively undifferentiated connective tissue cells.

The presence of double cells and cluster cells in the joint cartilage surrounding the defects at two and six weeks and furthermore the clear demarcation of the cartilage covering the peripheral parts of the defects from the fibrocartilage in the central regions, even at the late observations, should clearly indicate that the cartilage covering the peripheral parts of the defects was derived from the joint cartilage.

The morphological appearance of the cartilage created in these ways was definitely different from the cartilage seen in the defects into which osteochondral transplants (tr.pl. A) had been implanted, except for two defects at six and 14 months (p. 28 and Table 3).

Transplants D

Vitality

The remnants of devitalized cartilage contained, at all observation times, cells with approximately the same morphological appearance as the necrotic cartilage cells of the osteochondral transplants A. From six weeks on, the matrix was found to be relatively strongly stained with azophloxine (Figures 39, A, B and D, 41 A, 42 A). The devitalized cartilage stained more weakly with Alcian green than the cartilage of the vital transplants and than the joint cartilage (Figure 40).

Presence of connective tissue, formation of cartilage, ossification

At two weeks a connective tissue containing elongated, fusiformed cells appeared to have grown into the devitalized cartilage, which had been partly resorbed (Figure 38).

At six weeks a more marked resorption of the devitalized cartilage had taken place; large regions of the cartilage had been replaced by connective tissue and slightly fibrous cartilage (Figure 39, A, B and C).

The cells of this slightly fibrous cartilage bacame swollen and enchondral ossification was seen in the basal regions (Figure 39 D). (In some defects bone tissue also seemed to be formed directly on connective tissue.) In the superficial part of the defects the cartilage appeared to be small-celled, and there were neither vessels nor signs of ossification to be seen in these regions (Figure 39 B).

At twelve weeks the resorption of the devitalized cartilage and the ossification of the slightly fibrous cartilage was in general found to be still more advanded (Figure 41, A and C).

At six months large regions of the latter cartilage had obviously become ossified, but the ossification had still not been brought to an end (Figure 42 A). The cartilage of the most superficial zone had in some transplants undergone changes, making it more like hyaline cartilage than at the earlier observations (Figure 42, B and C).

At nine months the new vital cartilage was found to be almost completely ossified except in the superficial part of the defects. However, there were still considerable amounts of unresorbed devitalized cartilage in the central regions of the original defects.

At the last observation, 14 months, however, only scanty remnants of the devitalized cartilage were to be seen. Neither at this time of observation were there any signs of ossification of the cartilage of the most superficial zone. In one case bone tissue filled the defect up to a level slightly superficial to the basal level of the adjacent joint cartilage (Figure 43 A). In the basal part of the persisting cartilage of this region a zone resembling the calcified zone of the adjacent joint cartilage was seen, beyond which no signs of vascularization and ossification could be observed (Figure 43 B).

Changes within the surrounding joint cartilage

The defects tended to varying extents to be covered peripherally with a cartilage which was centrally demarcated by a narrow zone of necrotic cells. Although a relatively wide range of variation was

seen at each observation time, this tendency seemed to increase up to twelve weeks (Figures 39 A, 41 A).

Also at the later observations this type of cartilage covered the defects to varying extents. Some defects were covered only strictly peripherally, whereas others were almost completely covered by this type of cartilage (Figures 42 A and 43 C).

Double cells and cluster cells were especially abundant in this cartilage from six weeks on (Figures 39 B and 41 B).

Comments

Slightly fibrous cartilage was obviously formed within devitalized cartilage in regions where connective tissue was present at six weeks. The quantity of such cartilage increased at the expense of devitalized cartilage and ingrown connective tissue at the later observations. Hence it should be evident that the cartilage present at the later observations had also been formed by metaplasia from connective tissue, except the cartilage which covered the defects peripherally. The morphological appearance of the latter was quite similar to that of the cartilage which covered the peripheral parts of the defects in the controls C (p. 42). This cartilage obviously originated from the surrounding joint cartilage.

It should be noticed that the structure of the metaplastically formed cartilage did not differ significantly from the vital cartilage seen within the necrotic areas of the transplants A and B. However, it was quite different from the structure of the cartilage which had been evaluated as vital apophyseal cartilage following transplantation (p. 23 and Table 3).

DISCUSSION

Aim of study

The purpose of the present study was to investigate some of the changes that take place in apophyseal cartilage following autogenous transplantation to osteochondral defects of joints where it is subjected to the same types of mechanical stimuli as the joint cartilage of pressure epiphyses, i.e. intermittent pressure and friction against an opposite joint surface. It was of particular interest to shed light on the following points: the vitality of the cartilage following transplantation, the influence of the mechanical factors upon the cells and the matrix of the cartilage and upon the process of ossification, and especially on the question whether the mechanical stimuli would prevent ossification in the most superficial layers. Finally, it will be discussed if the findings suggest that autogenous transplantation of apophyseal cartilage could be applied as a method for the repair of joint defects in children.

Methodology

Before the results of the investigation can be discussed in relation to the problems mentioned above, however, the applied experimental model has to be assessed.

Transplantation of apophyseal cartilage on an osseous base was chosen with a view to a possible clinical application of such cartilage in restoring defects of joints, which, at least in some cases would demand transplantation not only of cartilage but also of underlying bone substance.

One necessary condition for drawing conclusions concerning the influence of the mechanical factors upon the cartilage is of course that the transplants to the joint surface of the femoral condyles were really subjected to the stimuli of intermittent pressure and friction and that the controls outside the joint surface were not.

During the examination of the operated joints these requirements were found to be fulfilled in most cases. The finding that

the menisci embraced a considerable part of the opposite joint surface does not indicate that the transplants have not been subjected to weight-bearing. In the dog the menisci are very broad and cover the major part of the tibial joint surface. They undoubtedly take part in the transmission of weight from femoral to tibial joint surfaces. These circumstances might, however, be of importance in the adjustment of the surface level of the transplants, since it seems reasonable to assume that the menisci are less resistant to pressure than the cartilaginous joint surface because of their consistency and because they are mobile to some extent.

The fact that the transplants were placed in the anterior part of the weight-bearing regions of the femoral condyles raises the question whether the transplants really were subjected to weight-bearing. The animals could possibly have avoided extending the knees completely. No such tendency could be registered, however, during the observation of the animals and no flexion contractures could be found in the operated joints. There is thus good reason to assume that the transplants were exposed to weight-bearing.

The location of the transplants in the anterior parts of the load-bearing surface, which had to be done for technical reasons, has, however, probably caused some damage to the superficial part of the transplants that protruded. The anterior part of the opposite joint surface must have been pushed against these transplants by each extension movement of the knee.

The surface of most transplants outside the joint surface, at the medial side of the medial femoral condyle, appeared to be level with that of the surroundings, and these transplants therefore have not to any appreciable extent been exposed to pressure. In some cases a slight protrusion was found, however. This has to be taken into consideration when the importance of the mechanical factors are discussed.

Another finding that also has to be discussed in connection with the comparison of the fate of the transplants in the two mentioned positions, is that most of the transplants outside the joint surface, in contrast to those within the joint surface, were separated from the joint cavity by a layer of connective tissue that had grown over the surface of the transplants.

The use of pure osseous transplants and devitalized cartilage implants in the defects of the joint surface appeared to be an appropriate means of helping to distinguish the changes caused by

ingrowing granulation tissue and changes within necrotic cartilage from those changes that took place in cartilage that had survived the transplantation. Devitalization by heating at 50°C was chosen as a method of devitalization because it has previously been shown that this method gives a reliable devitalization of cartilage tissue (Boström & Månsson 1953). This temperature does not induce shrinking of the cartilage, since mammalian collagen fibres start shrinking first at 64°C (Chvapil 1967). The probability that this method of devitalization will not give quite identical changes within the cells and the matrix compared to those caused by insufficient nutrition, does not imply that this method is unsuitable for the particular purpose of clarifying the morphological and autoradiographic criteria of cell necrosis, making the differentiation of the necrotic areas from the vital ones more reliable.

Will apophyseal cartilage remain vital after transplantation to osteochondral defects of joints?

The morphological findings concerning the vitality of the transplanted cartilage were confirmed by an autoradiographic study (Benum 1973). The morphological criteria of vitality should therefore be reliable.

Two weeks after the transplantation of the osteochondral fragments from the iliac crest to the osteochondral defects of the femoral condyles, the major part of the cartilage of the transplants was found to be vital. In the basal regions necrotic changes were found to varying extents. They included areas ranging in size from the central part of the growth plate to the whole of the growth plate and the basal central parts of the overlying cartilage. These necrotic regions always became narrower towards the joint surface and never extended into that part of the cartilage which corresponded to the joint cartilage of the surroundings. At the later observations there were generally no signs of further necrotizing of the transplants except in the peripheral regions of those transplants that protruded from the joint surface, where some necrosis and breakdown of the cartilage were seen. This necrosis was probably due to the mechanical dysfunction of the joint surface.

The vitality of the transplants was demonstrated among other things by the marked tendency towards regeneration of the growth plates from the remaining peripheral vital parts of the growth plates

and from the overlying residual cells as seen at six weeks' observations. Certainly, the proliferation of the cells of the re-established growth plates ceased earlier than it did in the growth plates of the iliac crest. The investigation revealed, however, that the cartilage of most of the transplants remained vital during the whole observation period.

At the later observations a steadily decreasing height and width of the necrotic areas were found. This does not mean, however, that the areas of necrosis in these transplants had originally been smaller than in the transplants studied after two weeks, but simply that the resorption of necrotic cartilage by the ingrowing connective tissue and the ossification of metaplastically formed cartilage had resulted in a reduction of the demonstrable necrotic areas. Similar resorption, metaplasia and ossification also took place in the cartilage which had been devitalized prior to implantation. From these implants it was further found that the metaplastically formed cartilage of the superficial part of the defects did not ossify, probably because of the mechanical stimuli to which it was subjected.

This raises the question whether the vital cartilage found at the later observations in the superficial parts of those defects which contained the non-devitalized cartilage transplants had also been formed by a similar metaplasia, and therefore did not represent transplanted cartilage. However, the marked difference in morphological appearance between this cartilage and the metaplastically formed cartilage in the control defects, and furthermore the fact that no signs of necrosis and metaplastic formation of cartilage were ever seen in the superficial zone of the non-devitalized cartilage, at the early observations, speak strongly against this possibility.

The striking morphological difference of this cartilage from the fibrous or hyaline cartilage found in those control defects which had been partly filled with osseous transplants also rejects the objection that cartilage formed by metaplasia of granulation tissue covering the surface of the transplants or by overgrowth of articular cartilage might have been misinterpreted as transplanted apophyseal cartilage.

Different theories could be suggested concerning the cause for the necrosis of the central and basal regions of the cartilaginous part of the transplants. Necrosis with a similar distribution has previously been found within transplants consisting of epiphyseal cartilage and adjacent metaphyseal bone following implantation

into both autogenous bone and soft tissues, and also following reimplantation (Enderlen 1899, Axhausen 1912, Heller 1914, 1918, Obata 1914, Ring 1955, Heikel 1960, Ryøppy 1965).

Such necrosis has been suggested to be a result of ischaemia.

It seems reasonable to suppose that ischaemia is also responsible for the basal and central necrosis of the apophyseal cartilage transplants of the present investigation. The diffusion of nutrients from the bone bed has probably not been sufficient to keep the central regions alive, in contrast to the more peripheral ones. It could be proposed that the osseous base of the transplants, by obstructing the diffusion of nutrients, might have contributed to the cessation of proliferation and to the necrosis of the cartilage cells of the growth plate. It has been shown, however, that the vitality of the cartilaginous part of the growth plate in pressure epiphyses does not depend on the metaphyseal blood supply but on the epiphyseal vessels alone (Trueta 1968). Hence this explanation seems to be unlikely.

The fact that the cartilage near the joint surface was generally vital suggests that this part of the transplants is nourished from the synovial fluid. The narrowing of the underlying central necrosis towards this zone further indicates that the combined nutrition from the synovial fluid and from the bone bed reduces the tendency of necrosis towards the joint surface of the transplants. It is harly likely that the vascularization of the cartilaginous part of the transplants played any role in the immediate preservation of the vitality of the cartilage following the transplantation because no definite ingrowth of vessels could be found in the vital cartilage regions at two weeks' observation. The necrosis of the most peripheral cartilage cells had obviously been caused by the preparation of the transplants.

The central regions of the cartilaginous part of the growth plates were obviously regenerated from the surviving peripheral parts and from vital reserve cells overlying the necrotic regions. At two week's observation, however, the ossification in the preserved or regenerated growth plates were found to be less developed than later on, when the osseous parts of the transplants were found to be better revascularized. This indicates that ossification probably could not take place before the osseous part of the transplants had been sufficiently revascularized and the necrotic cartilage regions had been penetrated by vessels.

The early cessation of the proliferation of the cells within the re-established growth plates is in accordance with the findings within transplanted epiphyseal plates, in which the proliferation also ceases prematurely (Heikel 1960). The explanation might be the same, i.e. that the reserve cell zone, due to the high rate of proliferation necessary for regeneration of the growth plate, exhausts its resources.

One possible contributory cause of the partial or total necrosis of the growth plates might be that the loosening between the cartilage and the metaphyseal bone, which occurred during the preparation or the implantation of some of the transplants, might have damaged the cartilage. It does not seem to be likely, however, that this factor has been of major importance to the necrosis, although it might have contributed to it, since such loosening occurred relatively rarely. A strong compression of the growth plate during the implantation of the transplants might also have been of some importance. Pressure and friction from the opposite joint surface cannot, however, be responsible for the necrosis, the distribution of necrosis being practically the same within the transplants outside the joint surface.

The possibility that the cells of the various regions within the apophyseal cartilage could differ with regard to their tendency to undergo necrosis following transplantation because of different metabolic activity and demands, cannot be rejected as a possible disposing factor for the necrosis registered in the growth plate. It seems unlikely, however, that such factors would also contribute to the necrosis of the central regions of the overlying cartilage.

The superficial necrosis and destruction of the peripheral parts seen in some of the protruding transplants after the longest observation times have to be attributed to the mechanical discrepancy caused by the protrusion of the surface of the transplants. Similar changes were not found in those transplants that were level with the surrounding joint surface.

Do the mechanical stimuli from the opposite joint surface induce changes of the cartilage, making it more like joint cartilage?

The cells of the most superficial zone of the weight-bearing transplants did not undergo hypertrophic changes. They remained small and very like articular cartilage cells in contrast to some cells

of the deeper zone and also in contrast to many cells of the transplants outside the joint surface. These facts suggest that intermittent pressure and friction may prevent the transformations of the cartilage cells. The registered difference between the cells of the superficial and the deeper zone of the transplants, however, might also be attributed to biological environmental differences. Only the cartilage of the deeper zone was surrounded by bone tissue, whereas the cells of the superficial zone were exposed to synovial fluid. Under all circumstances mechanical factors seem to be the most likely reason for the flattening of the most superficial cells, which was found in some transplants from six weeks on.

The matrix component appeared to remain hyaline and to be rich in acid mucopolysaccharides during all the observation periods, as evaluated by the Gomori and Alcian-green staining methods. No difference could be registered between the matrices of the superficial and of the deeper zone of the transplants. Neither could a comparison with the transplants outside the joint surface suggest that mechanical factors brought about specific morphological changes of the matrix, with the possible exception of the formation of a basal calcified zone, which will be discussed later. Certainly the matrix of the ossification zone in some of the transplants outside the joint surface appeared to be more strongly stained with Alcian-green than that of the weight-bearing transplants. These findings were inconsistent, however, and since the applied technique had not been planned for an exact study of the quantities of the acid mucopolysaccharides, no certain conclusions can be drawn from the histochemical findings.

Does the transplanted cartilage undergo complete ossification or does the process of ossification fail to appear in the superficial zone because of the mechanical influence of the opposite joint surface?

No ossification of the cartilage of the most superficial zone could at any time be observed in the transplants to the osteochondral defects of the femoral condyles. The ossification had, from six months onwards, frequently reached a level corresponding to that of the base of the surrounding joint cartilage or even somewhat higher. The findings were different, however, in the transplants to defects

outside the joint surface. Hence, the persistence of cartilage in the most superficial zone seems to be due to a real prevention of and not only a delay of the process of ossification. The finding that a calcified zone well demarcated by a cementing line, similar to that of the most basal cell layers of mature articular cartilage, had developed in such transplants at nine and fourteen months also strongly suggests that the remaining cartilage of these transplants would not have become ossified even if the observation time had been prolonged. The formation of a corresponding zone in articular cartilage is usually considered a sign that full maturity of the articular cartilage has been reached and that no further ossification will take place (Fawns & Landells 1953, Mankin 1963 b, Hodge & McKibbin 1969).

The findings that the ossification was far more extensive in the transplants outside the joint surface and in particular that the superficial zone of these transplants also underwent ossification to an increasing extent from twelve weeks on, furthermore strongly suggest that the mechanical stimuli from the opposite joint surface were responsible for the prevention of the ossification of the former transplants. Remnants of unossified cartilage could be observed, however, after 14 months, even in some of the transplants outside the joint surface. These remnants were found mainly in the superficial part of those transplants that protruded. It seems reasonable to assume that these transplants might have been subjected to some pressure and friction from the capsule and the retinaculi of the knee joint.

It could of course be objected that the existence of cartilage below the basal level of the surrounding joint cartilage in some weight-bearing transplants and the presence of some unossified cartilage in some of the transplants outside the joint surface at 14 months weaken the weight of evidence of the findings mentioned above. It should, however, be remembered that the persistence of cartilage is not dependent on the mechanical factors only but also on such factors as the height of the transplanted cartilage and the local conditions for vascularization. Besides, ossification was still seen at 14 months in the deeper parts of those weight-bearing transplants that had not been ossified all the way out to the superficial zone and also in those transplants outside the joint surface which still contained some remnants of unossified cartilage.

Some other possible contributory reasons for the different fate of the cartilage in the superficial part of the defects of the

two different locations, should also be discussed. Thus the influence of the different mechanical factors upon the process of ossification of apophyseal cartilage cannot be evaluated unless the vitality of the two types of transplants is adequate. Furthermore, there should be no doubt concerning the origin of the cartilage filling the defects. The defects should be filled with transplanted cartilage alone and not with cartilage formed by metaplasia or overgrowth from the surrounding articular cartilage. These conditions have been fulfilled in the present study.

Many of the transplants outside the joint surface were covered with a layer of connective tissue. This could possibly be a contributory cause of the ossification of the superficial zone of these transplants. No marked ingrowth of vascular connective tissue could be observed from this tissue, however, and in particular, no ossification was seen around vessels originating from the connective tissue covering the surface. There is therefore no evidence indicating that this connective tissue has contributed directly to the ossification of these transplants. The cartilage of these transplants is separated from the synovial fluid by the connective tissue layer. This could imply that biological environmental differences might be the reason why the transplants in the two locations differed with regard to their tendency to ossify. No report is available indicating that the synovial fluid should contain specific factors preventing ossification. Therefore such an explanation seems to be highly hypothetical.

Pressure has been found to favour chondrogenesis (Krompecher 1935, Glücksmann 1939, Hall 1968) and further, when combined with friction, also to prevent the ossification of cartilage (Krompecher & Goerttler 1938) even during conditions where synovial fluid is absent. These findings should also support the assumption that the mechanical factors, rather than the synovial fluid, are responsible for the prevention of ossification of the apophyseal cartilage transplants to the defects of the joint surfaces in this study.

The location of the donor site within the iliac crest is one factor that could possibly influence the time of appearance and the extent of the ossification of the transplanted cartilage. The fact that the secondary centre of ossification is first seen within the posterior part of the iliac crest could indicate that the cartilage within this region becomes mature for ossification earlier than the rest of the cartilage. Hence the cartilage from this region of the apophysis might also undergo ossification prior to

that from the anterior part following transplantation. It was shown, however, by the experiments performed, that the time of appearance and the extent of ossification of the transplanted cartilage were in no way dependent on the location of the donor site within the iliac crest. A possible factor of differently developed maturity of the cartilage should not therefore complicate the evaluation of the role of the mechanical factors in the prevention of ossification.

Theoretically the mechanical stimuli of intermittent pressure and friction might prevent the ossification of the superficial zone of the transplants in the joint surface of the femoral condyles in at least two ways. They might prevent the ossification either by directly influencing the cartilage cells to produce an unossifiable matrix or by inhibiting the vascularization of the cartilage by causing intermittent compression of the cartilage.

This study did not show any significant difference concerning the details of the ossification in the deeper part of the transplants within the joint surface from those of the transplants outside the joint surface. Ossification occurred in both types of transplants without any marked hypertrophic changes of the cartilage cells. Similar findings were observed in the iliac crest during the advanced stages of the secondary ossification. This indicates that the lack of swollen cells within the most superficial part of the weight-bearing transplants cannot be the primary reason why the cartilage of this zone does not ossify. On the ohter hand, the finding of a sharply demarcated calcified basal zone, beyond which no vascularization and ossification are seen, does not definitely prove that the lack of vascularization is the primary cause. The lack of ability of the matrix to ossify, induced by mechanical influence on the cartilage cells, might involve a lack of ability to produce a vascularization-stimulating principle. The formation of the well-demarcated calcified zone could, in that case, be the result of and not the cause of the arrest of the vascularization and the ossification.

It has been suggested that a low microenvironmental oxygen supply plays a role in the direction of the differentiation of the skeletal stem cells. Hypoxia possibly favours acid mucopolysaccharide synthesis, which determines the cells for chondrogenesis, whereas oxygen sufficiency determines the cells for osteogenesis (Hall 1969, 1970, 1971). A similar mechanism could possibly be responsible for the prevention of the ossification of the apophyseal cartilage in

the load-bearing defects in this study. Thus it has been found that acid mucopolysaccharide synthesis may be related to mechanical stress even when applied to developed cartilage (Akeson et al. 1958, Hall 1971) and furthermore that such compounds in one way or another may play a role during the process of ossification (Herring 1972). This possibility favours the theory that the ossification of the superficial part of the transplants is prevented by inhibition of the vascularization of the cartilage caused by intermittent compression. Even without the existence of such a mechanism, however, it seems likely that the mechanical stimuli might prevent the ossification directly by compressing the vessels, since the presence of vessels under all circumstances is essential in bone formation (Trueta 1968).

The height of the unossified zone in some of the weight-bearing transplants and also the height of the unossified fibro-cartilage in some of the control defects at 9 and 14 months were considerably lower than that of the surrounding joint cartilage. These findings could mean that other factors than mechanical ones are the reason for the persistence of unossified articular cartilage in the pressure epiphyses. They might, however, also possibly suggest that the mechanical properties of the cartilage of the pressure epiphyses might be different from those of the former types of cartilage and that this might be the reason why the vascularization is arrested and a well-demarcated calcified zone is formed at a lower level in the pressure epiphyses than in the apophyseal cartilage transplants and in the fibro-cartilage of the control defects.

The findings of the present study do not conclusively indicate which one of these explanations is the right one. Some evidence presented in previous investigations favours the opinion that the persistence of joint cartilage on the articular ends of pressure-epiphyses is due to genetical factors rather than mechanical ones (McKibbin & Holdsworth 1967). The findings of Bentley & Greer (1971) that cartilage produced by isolated epiphyseal cells following allotransplantation to defects of joints does not undergo ossification might, however, suggest that the mechanical factors might be of some importance.

The newly reported findings of Enneking & Horowitz (1972) that enchondral ossification may take place in articular cartilage of human joints following immobilization and in the articular cartilage of rabbits during immobilization under compression (Walcher 1972), might also support this view. Besides, these findings seem

to be highly interesting when compared to the findings of the present study concerning the fate of the transplanted apophyseal cartilage.

Do the macroscopical and histological findings after autogenous transplantation of apophyseal cartilage to osteochondral defects of joints in dogs suggest that transplantation of apophyseal cartilage could be applied as a method of repair of such defects in children?

The following conditions would preferably have to be fulfilled if transplantation of apophyseal cartilage were to be used in the repair of defects of joints: the method should enable the restoration of a joint surface covered with vital hyaline cartilage, the transplants should heal in with the surrounding joint cartilage, the reconstructed joint surface should be congruent with the opposite joint surface, and it should be able to resist irreversible deformation without causing damage to the opposite joint surface.

In the present study a vital hyaline cartilage persisted near the joint surface up to 14 months following transplantation. This cartilage was not only vital according to morphological criteria. Autoradiographic investigations also revealed that the chondrocytes synthesized sulphur-containing compounds which were secreted in the matrix (Benum 1973). The findings further indicated that this cartilage would probably not undergo ossification. It is, however, still an open question whether the cells of the cartilage would sustain the wear and tear of joint function over years without degenerating.

In larger transplants, the width of the central necrosis overlying the osseous base of the transplants would most likely be great, since it has been demonstrated that the diffusion of nutrients from the surrounding bone bed is sufficient to keep only the peripheral parts of the transplants alive. The vitality of the superficial zone of cartilage can probably be maintained by nutrition from the synovial fluid alone. Therefore the width of the transplants should not influence the vitality of the cartilage near the joint surface.

The height of the necrosis of the central part would most probably be increased by an increase of the height of the transplants. The reasons for this assumption are obvious from the

discussion about the possible ways of nutrition of the transplanted cartilage. Therefore, in patients, the height of transplanted cartilage would have to be kept to the minimum that would suffice to reconstruct the defect of the cartilage. The height of the osseous part of the transplant would have to be adjusted to fill the osseous part of the defect. A basal central necrosis would not, however, necessarily lead to an unsuccessful result. Necrotic cartilage might, as shown in this study, be resorbed by ingrowing connective tissue and finally be replaced by metaplastically formed cartilage that eventually could ossify.

Although there were some signs of proliferation both in the peripheral parts of the transplants and in the adjacent joint cartilage no union was seen to take place directly between the transplanted apophyseal cartilage and the joint cartilage. The apophyseal cartilage united to the joint cartilage only by means of granulation tissue. This granulation tissue was, like the granulation tissue in pure osteochondral defects of joints (for references see DePalma et al. 1966), transformed to fibrous or hyaline-like cartilage.

Such union never occurred when there was a very close contact between the transplant and the joint cartilage. This finding suggests that the granulation tissue does not develop in very narrow clefts. The cleft probably has to exceed a certain width to avoid being completely obstructed during compression of the cartilage at weight-bearing. The width necessary to achieve union by ingrowing granulation tissue was not determined in the present study, since this factor was not taken into consideration when the project was planned. Union was, however, always seen when the interjacent cleft between the transplant and the surrounding joint cartilage had accidentally been made wider than 0.3 mm, as evaluated by the microscopical investigations.

The question about the congruence of the joint surface depends on several factors: the growth potency of the transplants, the tendency of the transplants to be destroyed and the possibility of a tendency of the surrounding joint surface to collapse.

In the present study a slight protrusion (maximally 1.4 mm) of the transplants was found in some joints at twelve weeks. This tendency declined, probably because of the cessation of the proliferative activity of the cells within the growth plate. The fact that no protrusion was observed in regions where the transplanted cartilage had been united to the adjacent joint cartilage might

suggest that the congruence of the surface of the transplants could have been improved if a cleft of a sufficient width had been systematically created around the transplants down to the subchondral bone, thus favouring the process of union.

During the first twelve weeks of observation there were no signs of destruction or collapse of the reconstructed joint surface. At the later observations some of the protruding transplants had become frayed peripherally and in a few transplants the central areas of the surface also appeared to be affected. The extent to which a joint surface would be able to resist weight-bearing if a relatively larger part of it had to be replaced, and if the observation time had been considerably prolonged, cannot be concluded from the findings recorded.

The question whether a joint surface reconstructed by transplanted apophyseal cartilage would be able to resist deformation without causing damage to the opposite joint surface does not entirely depend on the factors already discussed. The factors of compressibility and elasticity of the reconstructed joint surface must also be taken into consideration. No attempts have been made during this study to assess these qualities by biomechanical methods. Judging from the histological findings, however, it seems that these qualities might be significantly different from those of the surrounding joint surface. Thus the height of the cartilage was considerably lower than that of the surrounding joint cartilage in some transplants at 14 months. Moreover, the trabecular structure of the subchondral bone of the reconstructed part of the joint surface was also found to be different from that of the surrounding bone. On the other hand, the structure of the subchondral bone tissue appeared to be similar to the normal subchondral bone structure in other transplants, suggesting that functional adaptation of the bone structure may take place.

From a comparison between the opposite joint surface corresponding to the different types of transplants applied in this study, hardly any information can be obtained about these problems, the recorded damage to the cartilage of the tibial joint surface being relatively limited in all groups.

A comparison of the findings of the present study with those of Ponseti et al. (1968), reveals that the histological structure and the process of ossification of the iliac crest do not seem to be significantly different in dogs from those in human beings. Therefore it seems reasonable to assume that the basic changes that

would take place in apophyseal cartilage following autogenous transplantation to osteochondral defects of joints in human beings would be principally the same as those seen in dogs. One condition for this, however, is that a limited function of the human joints during the first post-operative period, which would be expected because of the higher sensitivity to pain in human beings, would not play any major role for the nutrition of the transplanted cartilage.

Discussion of some secondary findings

Some of the secondary findings registered during the present investigation also deserve some attention, although they are not directly related to the primarily formulated problems of this study.

A tendency towards overgrowth of cartilage originating from the surrounding joint cartilage was found after two weeks in the control defects of the femoral condyles, into which bone tissue or bone and additionally devitalized cartilage had been implanted. After twelve weeks this overgrowth had in several cases almost completely covered the four-millimetre wide circular defects. The cartilage covering the peripheral parts of the defects was of a clearly hyaline nature, in contrast to the cartilage of the central parts of the defects, which appeared to be more like fibrous cartilage. Furthermore, the cartilage covering the peripheral parts of the defects was centrally demarcated by a narrow necrotic brim like the cartilage surrounding the defects at two weeks' observations, suggesting that the original marginal zone had been pushed centrally by proliferating cartilage. This necrotic zone had most probably been created by the preparation of the defects. These findings indicate that the cartilage which had covered the defects peripherally had originated from the surrounding joint cartilage and was not formed by metaplasia of granulation tissue. The high frequency of cluster cells in the same regions further demonstrated that the cells of the joint cartilage of immature dogs possess a capacity for proliferative activity, since cluster cells have previously been found to be indicative of cellular proliferation by autoradiographic investigations applying 3H-thymidine (Hulth et al. 1970).

These findings are in accordance with the results of previous investigations showing that an increased proliferative activity takes place in immature joint cartilage following surgical injuries (for references see Calandruccio & Gilmer 1962, and Mankin 1962 b). The findings concerning the degree of contribution of this proliferative activity to the repair of the osteochondral defects, however, conflict with the results of most previous investigations. Only a few investigators have reported that the articular cartilage may contribute significantly to the repair of subchondral defects of joints (Calandruccio & Gilmer 1962). The repair of such defects has been mainly attributed to the formation of cartilage from the granulation tissue deriving from the marrow spaces in the subchondral bone, the process of which has been described in detail by Shands (1931). Thus Mankin (1962 b) and DePalma et al. (1966) concluded that there was no evidence that the process of proliferation of the cartilage cells, in immature rabbit and dogs, contributes materially to the healing of the cartilage in subchondral defects of joints.

The reason for the conflicting findings concerning the degree of contribution of the articular cartilage itself to the repair of the osteochondral defects might be due to the possibility that the maturity of the experimental animals might have been different in the various investigations. The exact age of the dogs used in the experiments performed by DePalma et al. is not available. It is possible that the age of their dogs was higher than that of the puppies in the present study. It seems reasonable to assume that an eventual contribution of the articular cartilage to the repair of the defects could decline during the maturation of the animals. Thus it has been demonstrated (Mankin 1963 a) that the frequency of mitotic divisions of the cells of normal articular cartilage decreases as the animals grow older and finally ceases when full maturity is reached. On the other hand, Bennett & Bauer (1935) could not demonstrate any more complete repair of defects of 3-4 month-old puppies than in those of adult dogs.

Another possible reason for the different findings might be suggested, however. Thus Calandruccio & Gilmer (1962) suggested that the granulation tissue of subchondral defects might inhibit the proliferative activity of the articular cartilage cells because they registered a far more extensive proliferation of these cells in superficial defects not extending into the subchondral bone than in subchondral defects which became invaded by granulation tissue.

If this is true, the plugging of the defects with devitalized cartilage, or the partial filling of others with osseous implants, might have contributed to increased proliferation of the cartilage cells by reducing the ingrowth of granulation tissue into the defects. This explanation seems unlikely, however, because there was no difference between the two types of defects mentioned above with regard to overgrowth of cartilage originating from the surrounding joint cartilage in spite of the relative inability of the osseous transplants to prevent ingrowth of granulation tissue into the defects.

The other secondary finding of the present study which deserves some attention is that concerning the ability of devitalized apophyseal cartilage to induce cartilage and bone formation. Cartilage tissue was constantly formed within apophyseal cartilage devitalized by heating at 50°C and also occasionally seen in cartilage that had necrotized following transplantation. There seems to be no doubt that relatively undifferentiated connective tissue cells were transformed to cartilage cells under the influence of some inductive principle within the devitalized cartilage. Influence from the surrounding bone tissue cannot have been the reason for the registered metaplasia because cartilage formation was also seen even deep within the cartilage, where no immediate contact with bone tissue was found. It might, however, be implied that the ossification which occurred in the metaplastically formed cartilage was not necessarily due to a bone induction principle of the devitalized or newly formed cartilage but to some principle of the surrounding bone tissue, because this ossification was seen mainly peripherally adjacent to the bone tissue of the condyle.

These findings that devitalized apophyseal cartilage possesses the ability to induce cartilage formation and further that cartilage formed in this way tends to be replaced by bone have not to the author's knowledge, been previously registered. They are, however, in good accordance with the findings of Bridges & Pritchard (1958) that various other types of hypertrophying and normally ossifiable cartilage such as epiphyseal cartilage, cartilaginous callus and cartilage from the costo-chondral junctions devitalized in various ways are capable of inducing cartilage and bone formation.

Bridges & Pritchard claimed that the inductor demonstrated in hypertrophic cartilage might be of physiological significance, sine it has long been suspected that the close relationship between this type of cartilage and bone formation in normal development is due to the presence of inductors in the cartilage (Fell & Landauer 1935, Lacroix 1951). This view might also be supported by the findings of Urist & Adams (1968) that the basal layers containing the germinal cells of articular cartilage of young rats induced bone formation when allogenically implanted into the anterior chamber of the eye, in contrast to the more superficial layers and in contrast to full thickness transplants in senile rats.

The present investigation reveals that the superficial part of cartilage that has formed in osteochondral defects of joints by ingrowth of undifferentiated connective tissue into devitalized apophyseal cartilage implanted into the defects does not ossify. This might suggest that mechanical factors might also be of importance for the determination whether ossifiable cartilage really will become ossified during development.

SUMMARY AND CONCLUSIONS

The purpose of the present study was to investigate the basic histological changes that take place in cartilage of traction epiphyses (apophyseal cartilage) following autogenous transplantation to osteochondral defects of joints where it is subjected to the same types of mechanical stimuli as the joint cartilage of pressure epiphyses, i.e. intermittent pressure and friction against an opposite joint surface. It was of particular interest to clarify whether the cartilage would survive the transplantation and furthermore whether the mechanical stimuli from the opposite joint surface would induce changes of the cartilage, making it more like joint cartilage, and most of all whether the same stimuli would prevent the ossification of the most superficial layers of the cartilage. The study was performed to clarify these basic problems with a view to a possible application of osteochondral transplants from the apophysis of the iliac crest in the treatment of defects of joints in children.

Autogenous transplants of apophyseal cartilage from the iliac crest, including some parts of its underlying metaphyseal bone, were implanted into osteochondral defects of the load-bearing parts of the femoral condyles of 3-4-month-old dogs. The operations were performed before a secondary centre of ossification had appeared within the iliac crest. Similar transplants were placed at the medial side of the medial femoral condyle, outside the cartilaginous joint surface, to find which changes in the former transplants had been caused by the transplantation itself and which had been caused by the mechanical stimuli. Furthermore, apophyseal cartilage devitalized by heat at 50°C was implanted into ostechondral defects of the load-bearing parts of the femoral condyles, helping to distinguish the findings caused by necrosis from those caused by changes of cartilage still vital after transplantation. Finally, pure osseous grafts were transplanted from the iliac crest to similar defects, after removal of the overlying apophyseal cartilage. This was done to investigate further the role of the cartilaginous part of the transplants in the repair of the defects of the joints.

The results were examined by macroscopical examination and by histological methods at various intervals. The first examination was done two weeks after the operation and the last after 14 months.

The ossification process of the unutilized iliac crest was examined at the same intervals by radiological and histological methods. At the last observation 14 months postoperatively this ossification was found to be completed or only insignificant remnants of cartilage could be seen within the iliac crest.

From the findings recorded the following conclusions can be drawn:

Apophyseal cartilage from the iliac crest, transplanted on an osseous metaphyseal base, survives transplantation to osteochondral defects of joints, except the central parts of the growth plate and the central basal parts of the overlying cartilage, which undergo necrosis to a varying extent. The growth plate regenerates from the surviving peripheral parts and from the surviving parts of the reserve cell zone. The proliferation of the restored growth plate ceases prematurely, however, compared to that of the iliac crest. The most superficial part of the cartilaginous portion of the transplants never undergoes necrosis following transplantation.

The necrotic cartilage at the base of the cartilaginous portion of the transplants is resorbed by ingrowing connective tissue. This connective tissue undergoes metaplastic changes to cartilage which shows a strong tendency to be ossified.

The mechanical stimuli from the opposite joint surface induce many of the cells of the superficial cell layer to become flattened and to orientate their long axis parallel to the surface of the joint. They further prevent hypertrophic changes in the underlying cells of the superficial part of the cartilage. Finally, they cause the development of a demarcated calcified zone in the basal region of the persisting cartilage of transplants in mature animals.

The most superficial part of the load-bearing transplants does not undergo ossification in contrast to the more basal part and also in contrast to the cartilage of all the parts of the transplants outside the joint surface. Although the height of this zone was found to be lower than that of the surrounding joint cartilage in some mature animals, the findings strongly suggest that the mechanical stimuli from the opposite joint surface prevent the ossification of the most superficial part of the former transplants. The formation of a clearly demarcated calcified zone, in and beyond which no ossification seems to take place, further indicates that the cartilage would have remained unossified in these transplants even if the observation period had been prolonged.

By using the method described it has been shown that it is

possible to reconstruct a joint surface consisting of vital hyaline cartilage in dogs. It has further been shown that this cartilage is connected to the surrounding joint cartilage by an ingrowing granulation tissue, provided that a cleft is created around the transplant all the way down to subchondral bone. This granulation tissue is transformed to cartilage, which finally builds a bridge between the transplanted cartilage and the surrounding joint cartilage. The growth of the transplants exceeded that of the joint cartilage in some cases and this resulted in a slight protrusion of some transplants. Union between the transplanted cartilage and the joint cartilage seems to prevent such protrusion.

It is still too early to conclude that a joint surface reconstructed in this way might resist the wear and tear of the joint function over the years, even if it is possible to reconstruct a completely congruent joint surface.

Because the histological structure and the ossification process of apophyseal cartilage of the iliac crest seem to be almost identical in dogs and human beings, it seems reasonable to assume that the basic changes observed after autogenous transplantation of such cartilage to osteochondral defects of joints in dogs would also take place in human beings.

An increase of the width of the transplants, which would be necessary if the method were to be applied to human beings, should not influence the vitality of the most superficial part of the transplants, because this part seems to be sufficiently nourished by the synovial fluid. However, it would most probably cause increased necrosis of the basal part of the transplanted cartilage unless care were taken not to transplant cartilage of considerably greater thickness than that of the joint cartilage.

Conclusions from some secondary findings

The cartilage cells at the margin of osteochondral defects of joints partially filled with osseous grafts or completely filled with devitalized apophyseal cartilage possess a marked capacity to proliferate in 3-4-month-old dogs. This proliferation contributes significantly to the healing of the defects, but never restores the joint cartilage completely over the 4 mm wide defects. In the central parts of these defects, fibrous cartilage is formed by

metaplasia of undifferentiated connective tissue.

Apophyseal cartilage, having been transplanted to osteochondral defects of joints after devitalization by heating at 50°C, produces cartilage induction on ingrowing undifferentiated connective tissue. The cartilage formed by such induction is ossifiable, but the mechanical stimuli of joint function prevent the ossification in the superficial part of the defects. Whether this cartilage possesses a bone induction principle of its own, or whether the ossification depends on the surrounding bone tissue, cannot, however, be decided upon unless implantation of devitalized cartilage to soft tissues is performed.

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Table 1. Ossification of the iliac crest.
Radiographic investigation.

Observation period	Experimental animal no.	Presence of a secondary centre of ossification			Completed ossification		
		Cr 1	Cr 2	Cr 3	Cr 1	Cr 2	Cr 3
2 weeks	24	-	-	-	-	-	-
	25	-	-	-	-	-	-
	26	-	-	-	-	-	-
	28	-	-	-	-	-	-
6 weeks	19	-	-	-	-	-	-
	20	-	-	-	-	-	-
	21	-	-	-	-	-	-
	22	+	-	-	-	-	-
12 weeks	13	+	-	-	-	-	-
	15	+	+	-	-	-	-
	16	+	-	-	-	-	-
	18	+	-	-	-	-	-
6 months	29	+	+	-	-	-	-
	30	+	+	-	-	-	-
	6	+	+	-	-	-	-
	8	+	+	-	-	-	-
9 months	7	+	+	+	-	-	-
	9	+	+	+	+	+	+
	33	+	+	-	-	-	-
	34	+	+	-	-	-	-
14 months	11	+	+	+	+	+	+
	12	+	+	+	+	+	+
	14	+	+	+	+	+	+
	17	+	+	+	+	+	+

Cr 1: Posterior third of the iliac crest
Cr 2: Middle " "
Cr 3: Anterior " "

Table 2. Histological investigation of the iliac crest.

Observation period	Experimental animal no.	Presence of growth plate			Presence of secondary centre of ossification			Completed ossification		
		Cr 1	Cr 2	Cr 3	Cr 1	Cr 2	Cr 3	Cr 1	Cr 2	Cr 3
2 weeks	24	+	+	+	-	-	-	-	-	-
	25	+	+	+	-	-	-	-	-	-
	26	+	+	+	-	-	-	-	-	-
	28	+	+	+	-	-	-	-	-	-
6 weeks	19	+	+	+	-	-	-	-	-	-
	20	+	+	+	-	-	-	-	-	-
	21	+	+	+	-	-	-	-	-	-
	22	+	+	+	+	-	-	-	-	-
12 weeks	13	+	+	+	+	-	-	-	-	-
	15	+	+	+	+	-	-	-	-	-
	16	+	+	+	+	-	-	-	-	-
	18	+	+	+	-	-	-	-	-	-
6 months	29	+	+	+	+	-	-	-	-	-
	30	+	+	+	+	+	-	-	-	-
	6	+	+	+	+	+	-	-	-	-
	8	+	+	+	+	+	-	-	-	-
9 months	7	(+)	+	(+)	+	+	+	-	-	-
	9	-	-	-	+	+	+	⊕	⊕	-
	33	(+)	(+)	-	+	+	-	-	-	-
	34	(+)	+	(+)	+	+	-	-	-	-
14 months	11	-	-	-	+	+	-	+	⊕	-
	12	-	-	-	+	+	+	+	-	+
	14	-	-	-	+	+	+	+	+	⊕
	17	-	-	-	+	+	+	+	+	+

Explanation (+): No regular columnar or cluster arrangements but still scattered clusters to be seen.

⊕ = only a small islet of cartilage left.

Table 3. Transplants A.

Presence of necrotic cartilage.

Obs. period	Tr.pl. no.	Zones a	b	c	growth plate	Obs. period	Tr.pl. no.	Zones a	b	c	growth plate
2 weeks	24-2	-	-	+	+++	6 months	29-2	-(a.c.)	-(a.c.)	-	-
	24-5	-	-	-	++		29-5	-	-	-	-
	25-2	-	-	++	+++		30-2	+(f)	-	-	-
	25-5	-	-	+	+++		30-5	+(f)	-(f)	-	-
	26-2	-	-	-	+++		6-2	-(f.c.)	-(f.c.)	+	-
	26-5	-	-	+	+++		6-5	-	-	+	-
	28-2	connect. tissue	-	-	++++		8-2	-	-	-	-
	28-5	-	-	-	++++		8-5	-	-	-	-
6 weeks	19-2	-	-	++	+	9 months	7-2	-	-	-	-
	19-5	-	-	-	+		7-5	-	-	-	-
	20-2	-	-	-	++		9-2	-	-	-	-
	20-5	-	-	+	-		9-5	-	-	+	-
	21-2	-	-	+	-		33-2	-	-	-	-
	21-5	-	-	+	-		33-5	-	-	-	-
	22-2	-	-	+	-		34-2	-(f)	-(f)	-	-
	22-5	-	-	+	-		34-5	-(f)	-(f)	-	-
12 weeks	13-2	Sections not available				14 months	11-2	-(f)	-(f)	+	-
	13-5	-	-	+	-		11-5	-	-	+	-
	15-2	-	-	+	-		12-2	-(f.c.) -(a.c.)	-(f.c.) -(a.c.)	-	-
	15-5	-	-	-	-		12-5	-(f.c.)	-(f.c.)	-	-
	16-2	-	-	+	+		14-2	-	-	-	-
	16-5	-	-	+	-		14-5	Sections not available			
	18-2	-	-	-	-		17-2	+(f)	+(f)	-	-
	18-5	-	-	+	-		17-5	-	-	-	-

Explanation: (n = necrotic area)

- : no observable necrosis

\+ : $n < \frac{1}{3}$ of the original area of the zone

++ : $\frac{1}{3} < n < \frac{2}{3}$ of the original area of the zone

+++ : $\frac{2}{3} < n < \frac{3}{3}$

++++: complete necrosis

f = fragmentation

f.c.= fibrocartilage

a.c.= artic. cartilage

Table 4. Transplants A.
Presence of vital growth plates.

Obs. period	Tr.pl. no.	Grading	Obs. period	Tr.pl. no.	Grading
2 weeks	24-2	+	6 months	29-2	-
	24-5	++		29-5	-
	25-2	+		30-2	-
	25-5	+		30-5	-
	26-2	+		6-2	-
	26-5	+		6-5	-
	28-2	-		8-2	-
	28-5	-		8-5	-
6 weeks	19-2	++	9 months	7-2	-
	19-5	++++		7-5	-
	20-2	++++		9-2	-
	20-5	+++		9-5	-
	21-2	++++		33-2	-
	21-5	++++		33-5	-
	22-2	++++		34-2	-
	22-5	++++		34-5	-
12 weeks	13-2	sections not available	14 months	11-2	-
	13-5	-		11-5	-
	15-2	-		12-2	-
	15-5	++++		12-5	-
	16-2	+		14-2	-
	16-5	+		14-5	sections not available
	18-2	++++		17-2	-
	18-5	-		17-5	-

Explanation: (w = width of vital growth plate present)

- : no signs of vital growth plate
+ : $0 < w < \frac{1}{3}$ of the width of the transplant
++ : $\frac{1}{3} < w < \frac{2}{3}$ " " " " " "
+++ : $\frac{2}{3} < w < \frac{3}{3}$ " " " " " "
++++ : $w = \frac{3}{3}$ " " " " " "

Table 5. Transplants A.

Occurrence of slightly swollen cells.

Obs. period	Tr.pl. no.	Zones a	b	c	Obs. period	Tr.pl. no.	Zones a	b	c
2 weeks	24-2	-	-	-	6 months	29-2	n.a.	n.a.	-
	24-5	-	-	-		29-5	-	-	+
	25-2	-	-	-		30-2	-(f)	-(f)	+
	25-5	-	-	-		30-5	-(f)	-(f)	+
	26-2	-	-	-		6-2	-	-	+
	26-5	-	-	-		6-5	-	-	-
	28-2	n.a.	-	-		8-2	-	-	-
	28-5	-	-	-		8-5	-	-	+
6 weeks	19-2	-	-	-	9 months	7-2	-	-	-
	19-5	-	-	-		7-5	-	-	+
	20-2	-	-	-		9-2	-	+	+
	20-5	-	-	+		9-5	-	-	c.o.
	21-2	-	-	-		33-2	-	c.o.	c.o.
	21-5	-	-	+		33-5	-	-	-
	22-2	-	-	-		34-2	-(f)	-(f)	-
	22-5	-	-	-		34-5	-(f)	-(f)	-
12 weeks	13-2	Sections not available			14 months	11-2	-(f)	+(f)	+
	13-5	-	-	+		11-5	-	+	+
	15-2	-	+	+		12-2	n.a.	n.a.	+
	15-5	-	-	+		12-5	n.a.	n.a.	c.o.
	16-2	-	-	-		14-2	-	c.o.	c.o.
	16-5	-	-	-		14-5	Sections not available		
	18-2	-	-	+		17-2	-(f)	-(f)	c.o.
	18-5	-	-	-		17-5	-	c.o.	+

Explanation:

n.a. = no cartilage of apophyseal origin

f = fragmentation

c.o. = completely ossified

Table 6. Transplants A.
Occurrence of "lining cells".

Obs. period	Tr.pl. no.	"Lining cells"	Obs. period	Tr.pl no.	"Lining cells"
2 weeks	24-2	-	6 months	29-2	n.a.
	24-5	-		29-5	n.a.
	25-2	-		30-2	- (f)
	25-5	-		30-5	- (f)
	26-2	-		6-2	n.a.
	26-5	-		6-5	-
	28-2	n.a.		8-2	-
	28-5	-		8-5	-
6 weeks	19-2	-	9 months	7-2	-
	19-5	-		7-5	+
	20-2	-		9-2	-
	20-5	+		9-5	+
	21-2	+		33-2	+
	21-5	+		33-5	-
	22-2	-		34-2	- (f)
	22-5	+		34-5	- (f)
12 weeks	13-2	Sections not available	14 months	11-2	- (f)
	13-5	-		11-5	+
	15-2	-		12-2	n.a.
	15-5	+		12-5	n.a.
	16-2	-		14-2	-
	16-5	-		14-5	Sections not available
	18-2	+		17-2	- (f)
	18-5	-		17-5	+

Explanation: n.a. = no cartilage of apophyseal origin
f = fragmentation

Table 7. Transplants A.

Fate of necrotic cartilage.

Obs. period	Tr.pl. no.	Presence of connect. tissue	Meta-plasia	New carti-lage	Ossifi-cation of new cart.	Obs. period	Tr.pl. no.	Presence of connect. tissue	Meta-plasia	New carti-lage	Ossifi-cation of new cart.
2 weeks	24-2	+	-	-		6 months	29-2	- *		-	
	24-5	-		-			29-5	- *		-	
	25-2	+	-	-			30-2	-		-	
	25-5	-		-			30-5	-		-	
	26-2	+	-	-			6-2	-		+	-
	26-5	+	-	-			6-5	-		-	
	28-2	+	-	-			8-2	- *		-	
	28-5	+	-	-			8-5	- *		-	
6 weeks	19-2	+	+	+	+	9 months	7-2	- *		-	
	19-5	-		-			7-5	- *		-	
	20-2	+	-	-			9-2	- *		-	
	20-5	-		-			9-5	-		-	
	21-2	-		+	+		33-2	- *		-	
	21-5	-		-			33-5	- *		-	
	22-2	-		-			34-2	- *		-	
	22-5	-		-			34-5	- *		-	
12 weeks	13-2	Sections not available				14 months	11-2	-		-	
	13-5	-		+	+		11-5	-		-	
	15-2	-		-			12-2	- *		+	-
	15-5	- *		-			12-5	- *		+	-
	16-2	-					14-2	- *		-	
	16-5	-		+	+		14-5	Sections not available			
	18-2	- *		-			17-2	-		-	
	18-5	-		-			17-5	- *		-	

Explanation: See text p. 38

* = no observable necrotic areas present (compare to Table 3).

Table 8. Transplants A.
Extent of ossification.

Obs. period	Tr.pl. no.	Zones a	b	c	Growth plate	Obs. period	Tr.pl. no.	Zones a	b	c	Growth plate
2 weeks	24-2	-	-	-	-	6 months	29-2	n.a.	n.a	++	-
	24-5	-	-	-	+		29-5	-	-	++	-
	25-2	-	-	-	-		30-2	-(f)	-(f)	++	-
	25-5	-	-	-	-		30-5	-(f)	-(f)	+++	-
	26-2	-	-	-	-		6-2	-	-	++	-
	26-5	-	-	-	+		6-5	-	-	++	-
	28-2	n.a.	-	-	-		8-2	-	-	++	-
	28-5	-	-	-	-		8-5	-	-	+++	-
6 weeks	19-2	-	-	+	++	9 months	7-2	-	-	++	-
	19-5	-	-	++	++++		7-5	-	-	+	-
	20-2	-	-	++	++		9-2	-	-	++	-
	20-5	-	-	+	+++		9-5	-	-	++++	-
	21-2	-	-	++	++++		33-2	-	++++	++++	-
	21-5	-	-	+	++++		33-5	-	-	+	-
	22-2	-	-	+	++++		34-2	-(f)	-(f)	+	-
	22-5	-	-	++	++++		34-5	-(f)	-(f)	++	-
12 weeks	13-2	Sections not available				14 months	11-2	-(f)	-(f)	+	-
	13-5	-	-	+++	-		11-5	-	-	+++	-
	15-2	-	-	+++	-		12-2	n.a.	n.a.	+++	-
	15-5	-	-	+	++++		12-5	n.a.	n.a.	++++	-
	16-2	-	-	+	+		14-2	-	++++	++++	-
	16-5	-	-	+	+		14-5	Sections not available			
	18-2	-	-	+	++++		17-2	-(f)	-(f)	++++	-
	18-5	-	-	+	-		17-5	-	++++	+++	-

Explanation: (for zone a, b and c):

+: 0 < ossified area < 1/3 of the original area of the zone

++: 1/3 < ossified area< 2/3 of the original area of the zone

+++: 2/3 < ossified area < 3/3 of the original area of the zone

++++: the cartilage of the zone is completely ossified

n.a.: no cartilage of apophyseal origin

f : fragmentation

For the growth plate: The grading indicates the width of the ossifying area out of the total width of the transplant.

Table 9. Transplants B.

Presence of necrotic cartilage.

Obs. period	Tr.pl. no.	Zones a	b	c	Growth plate	Obs. period	Tr.pl. no.	Zones a	b	c	Growth plate
2 weeks	24-3	-	-	+	++++	6 months	29-3	-	-	+	-
	24-6	-	-	+	++		29-6	+	+	+	-
	25-3	-	-	+	++		30-3	-	-	-	-
	25-6	-	-	+++	-		30-6	-	-	-	+
	26-3	-	-	+	++		6-3	+	+	+	-
	26-6	-	-	-	++++		6-6	-	-	-	-
	28-3	-	-	-	++++		8-3	-	-	-	-
	28-6	-	-	++	++++		8-6	-	-	+	-
6 weeks	19-3	-	-	+	-	9 months	7-3	-	-	-	-
	19-6	-	-	+	+		7-6	-	-	+	-
	20-3	-	-	-	-		9-3	-	-	+	-
	20-6	-	-	+	+		9-6	-	-	+	-
	21-3	-	-	-	-		33-3	-	-	-	-
	21-6	-	-	-	-		33-6	-	-	-	-
	22-3	-	-	-	+++		34-3	-	-	+	-
	22-6	-	-	-	-		34-6	++	-	-	-
12 weeks	13-3	-	-	-	-	14 months	11-3	+	+	+	-
	13-6	+	-	-	-		11-6	-	+	+	-
	15-3	+	+	-	-		12-3	+	+	-	-
	15-6	-	-	+	-		12-6	+	+	-	-
	16-3	-	-	+	+		14-3	-	-	-	-
	16-6	+	+	++	-		14-6	-	-	-	-
	18-3	+	-	-	-		17-3	-	-	-	-
	18-6	++	+	-	-		17-6	-	-	-	-

Explanation: See Table 3.

Table 10. Transplants B.

Presence of vital growth plates.

Obs. period	Tr.pl. no.	Grading	Obs. period	Tr.pl. no.	Grading
2 weeks	24-3	-	6 months	29-3	++
	24-6	++		29-6	+
	25-3	++++		30-3	-
	25-6	+		30-6	-
	26-3	++		6-3	-
	26-6	-		6-6	-
	28-3	-		8-3	-
	28-6	-		8-6	-
6 weeks	19-3	+	9 months	7-3	-
	19-6	++++		7-6	-
	20-3	-		9-3	-
	20-6	+++		9-6	-
	21-3	++++		33-3	-
	21-6	++		33-6	-
	22-3	++++		34-3	-
	22-6	++++		34-6	-
12 weeks	13-3	-	14 months	11-3	-
	13-6	-		11-6	-
	15-3	-		12-3	-
	15-6	-		12-6	-
	16-3	++		14-3	-
	16-6	-		14-6	-
	18-3	++		17-3	-
	18-6	-		17-6	-

Explanation: See Table 4.

Table II. Transplants B.

Fate of necrotic cartilage.

Obs. period	Tr.pl. no.	Presence of connect. tissue	Meta-plasia	New carti-lage	Ossifi-cation of new cart.	Obs. period	Tr.pl. no.	Presence of connect. tissue	Meta-plasia	New carti-lage	Ossifi-cation of new cart.
2 weeks	24-3	+	-	-		6 months	29-3	-		-	
	24-6	+	-	-			29-6	+	-	-	
	25-3	+	-	-			30-3	- *		-	
	25-6	+	-	-			30-6	-		-	
	26-3	-		-			6-3	-		-	
	26-6	-		-			6-6	- *		-	
	28-3	+	-	-			8-3	- *		-	
	28-6	+	-	-			8-6	-		+	-
6 weeks	19-3	-		+	+	9 months	7-3	- *		-	
	19-6	+	-	-			7-6	-		-	
	20-3	- *		-			9-3	-		+	-
	20-6	-		-			9-6	-		+	-
	21-3	- *		-			33-3	- *		-	
	21-6	- *		-			33-6	- *		-	
	22-3	-		-			34-3	-		-	
	22-6	- *		-			34-6	-		-	
12 weeks	13-3	- *		-		14 months	11-3	-		-	
	13-6	+	-	-			11-6	-		-	
	15-3	-		-			12-3	-		-	
	15-6	-		-			12-6	-		-	
	16-3	-		-			14-3	- *		-	
	16-6	+	-	+	-		14-6	- *		-	
	18-3	-		-			17-3	- *		-	
	18-6	+	-	-			17-6	- *		-	

Table 12. Transplants B.

Extent of ossification.

Obs. period	Tr.pl. no.	Zones a	b	c	Growth plate	Obs. period	Tr.pl. no.	Zones a	b	c	Growth plate
2 weeks	24-3	-	-	-	-	6 months	29-3	-	-	++	++
	24-6	-	-	-	++		29-6	-	++	+++	+
	25-3	-	-	+	++++		30-3	++++	++++	++++	-
	25-6	-	-	-	-		30-6	-	-	++++	-
	26-3	-	-	+	++		6-3	-	-	+++	-
	26-6	-	-	-	-		6-6	-	-	++++	-
	28-3	-	-	+	-		8-3	+++	++++	++++	-
	28-6	-	-	+	-		8-6	-	-	+	-
6 weeks	19-3	-	-	-	+	9 months	7-3	++++	++++	++++	-
	19-6	-	-	++	++++		7-6	++++	++++	++++	-
	20-3	-	-	+	-		9-3	-	-	++	-
	20-6	-	-	-	+++		9-6	++++	++++	++	-
	21-3	-	-	+	++++		33-3	-	-	+	-
	21-6	-	-	+++	++		33-6	+++	+++	+++	-
	22-3	-	-	-	++++		34-3	-	-	+	-
	22-6	-	-	+	++++		34-6	+	++++	++++	-
12 weeks	13-3	-	-	+++	-	14 months	11-3	+++	++	+++	-
	13-6	+++	++++	++++	-		11-6	+	+	+++	-
	15-3	+	++	++	-		12-3	-	-	+++	-
	15-6	-	-	+	-		12-6	+++	+++	++++	-
	16-3	-	-	+	++		14-3	+	+	+++	-
	16-6	-	-	+	-		14-6	++++	++++	++++	-
	18-3	-	-	++	++		17-3	+	++++	++++	-
	18-6	+	++	+++	-		17-6	++++	++++	++++	-

Explanation: See Table 8.

Figure 1.

A. Position of defects.

B. Relation between donor site and implantation site.

Tr.pl. A:	Donor - and implantation site nos.	2	and 5
Tr.pl. B:	" "	3	and 6
Tr.pl. C:	" "	1	and 4
Tr.pl. D:	" "	1	and 4

Tr.pl. A: Osteochondral transplants.
Tr.pl. B: Osteochondral transplants.
Tr.pl. C: Osseous transplants.
Tr.pl. D: Osseous transplant and devitalized cartilage

b = biopsy

A

B

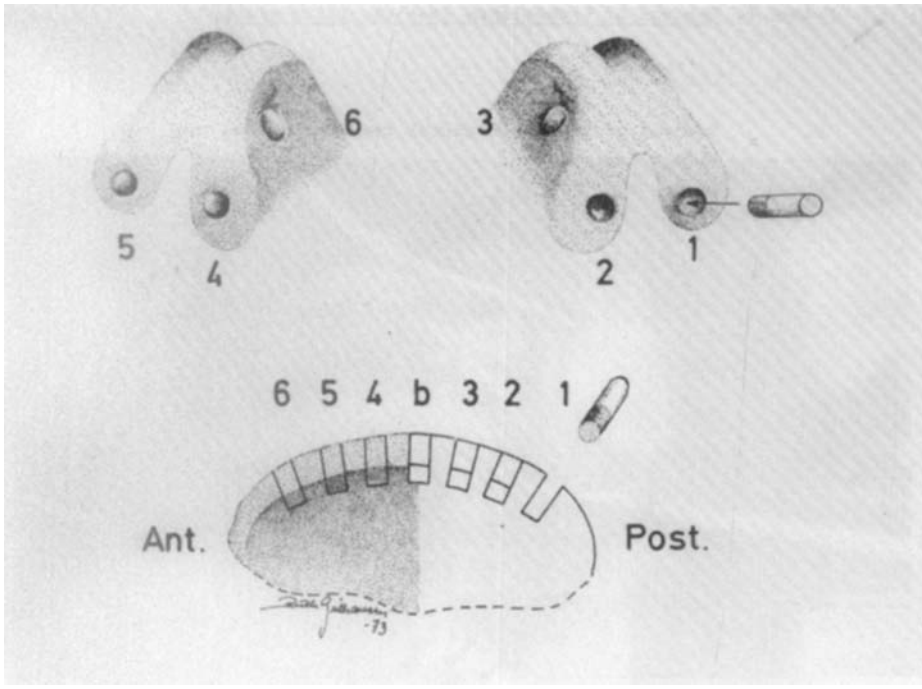
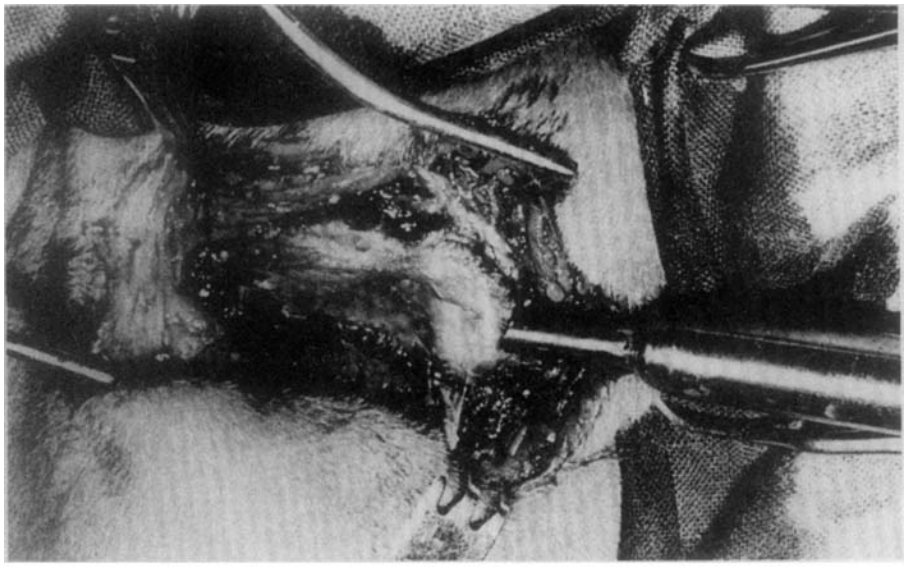


Figure 2. Removal of transplants from the iliac crest.

A. Removal of an osteochondral transplant from the left iliac crest.

B. Removed transplants. The height of the osseous part has not been adjusted.

A



B

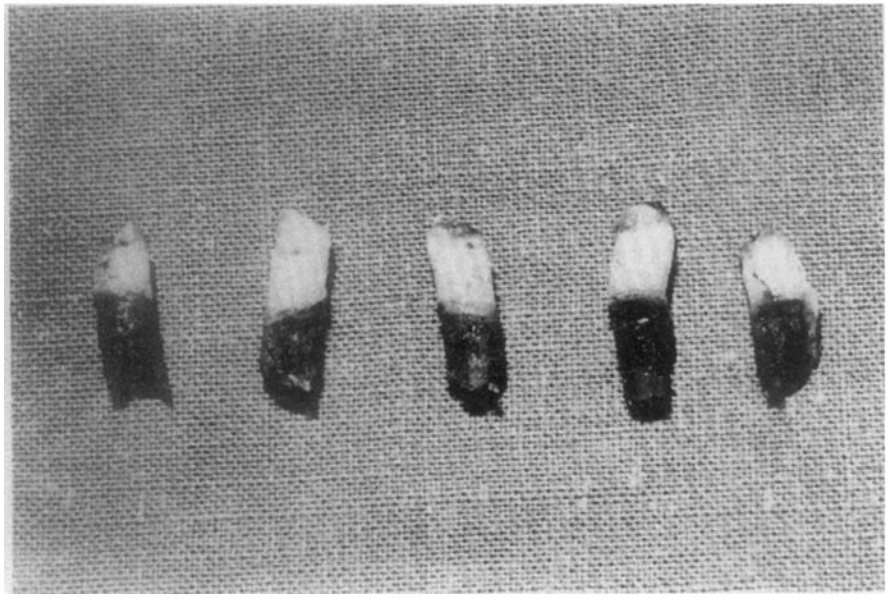
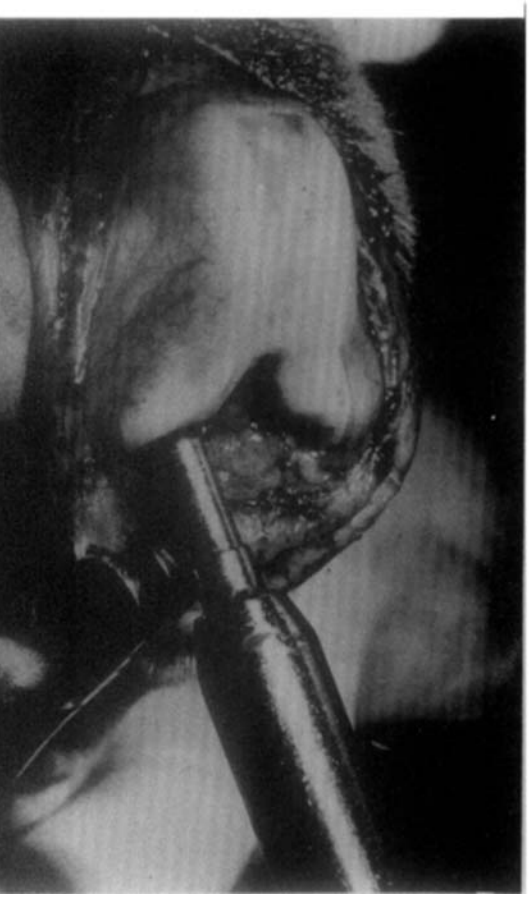


Figure 3. Insertion of transplants.

A. Preparation of defects in the joint surface of the femoral condyles by means of a punch. (The central part of the defects was prepared by use of a drill through the inserted punch.)

B. Left knee after insertion of an osteochondral transplant in the joint surface of the medial femoral condyle and at the side of the condyle, outside the joint surface. The perichondrial tissue and its underlying cartilage has been excised after insertion of the transplants. The defect of the lateral femoral condyle has been partially filled with osseous transplant.



A

B

Figure 4. X-rays pictures showing the extension of the secondary centre of ossification of the iliac crest at various times of observation.

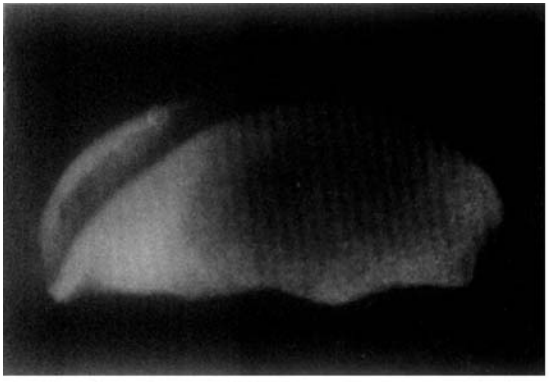
A. Observation period: 12 weeks.

The secondary centre of ossification is still confined to the posterior third of the iliac crest.

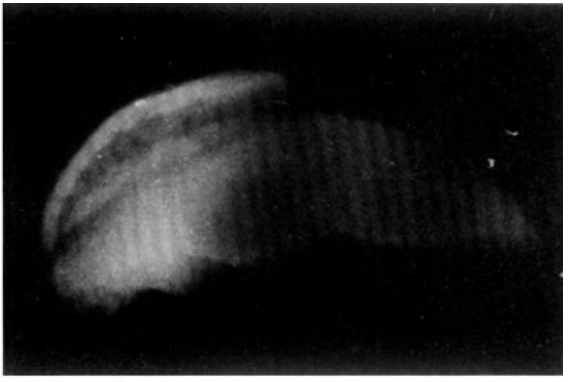
B. Observation period: 6 months.
The secondary centre of ossification has extended into the middle third of the apophysis.

C. Observation period: 9 months.
Secondary ossification takes place along all the crest.

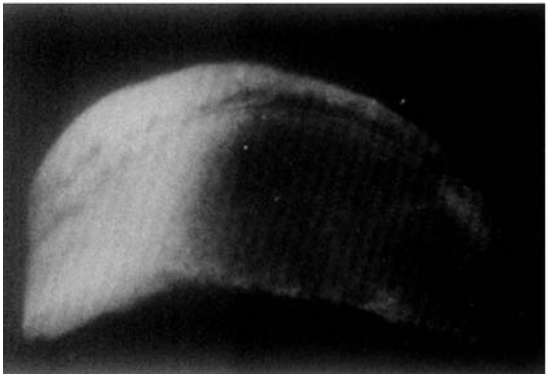
D. Observation period: 14 months.
The secondary centre of ossification has fused to the metaphysis.



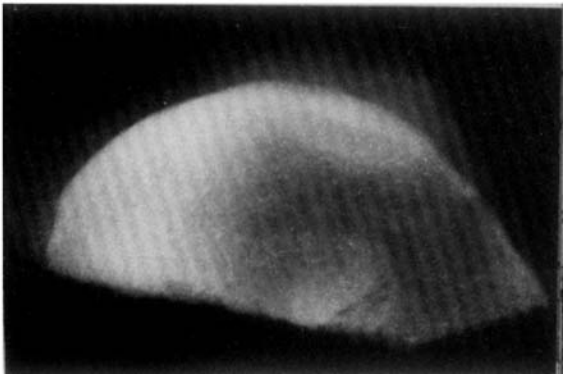
A



B



C



D

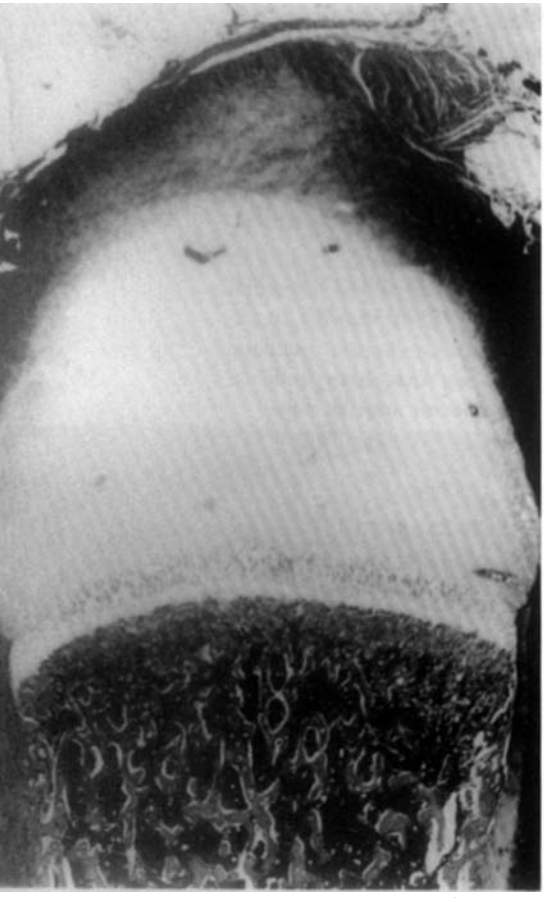
Figure 5. Histological sections from the iliac crest. Observation period: 6 weeks (4½-month-old dog). (Before the appearance of a secondary centre of ossification).

A. General view, cross section. Haemalun-azophloxine-saffron, x 15)

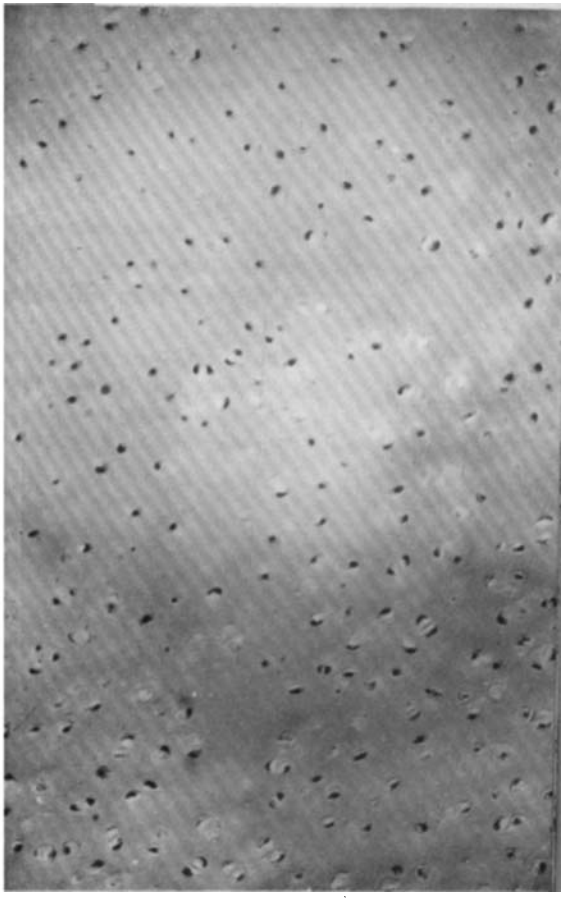
The apophysis consists of hyaline cartilage surrounded by a perichondrial layer into which muscle fibres are attached. Some vessels are seen peripherally within the cartilage. A growth plate is seen in the basal region.

B. Enlarged detail from the central part of the hyaline cartilage of Figure 5 A. (Haemalun-azophloxine-saffron, x 150)

Most of the cells are single and contain round or oval nuclei; some "double cells" are seen.



A

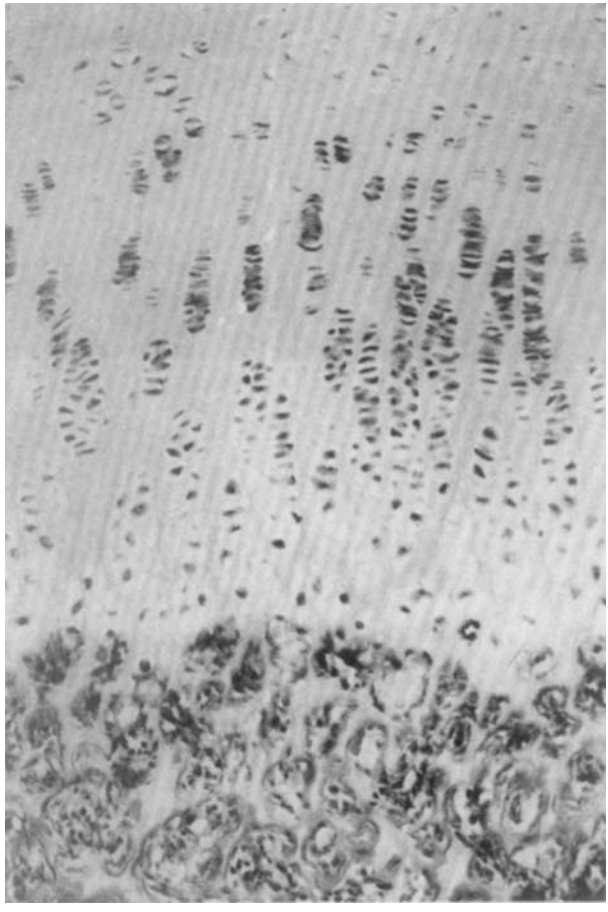


B

Figure 6. Histological sections from the iliac crest. Observation period: 6 weeks (4½-month-old dog). (From the same specimen as shown in Figure 5).

A. Growth plate, cross section. (Haemalun-azophloxine-saffron, x 150)

The cartilage cells are arranged in regular columns. Towards the base of these columns the cells undergo a progressive swelling. The bottom area is penetrated by small vessels.

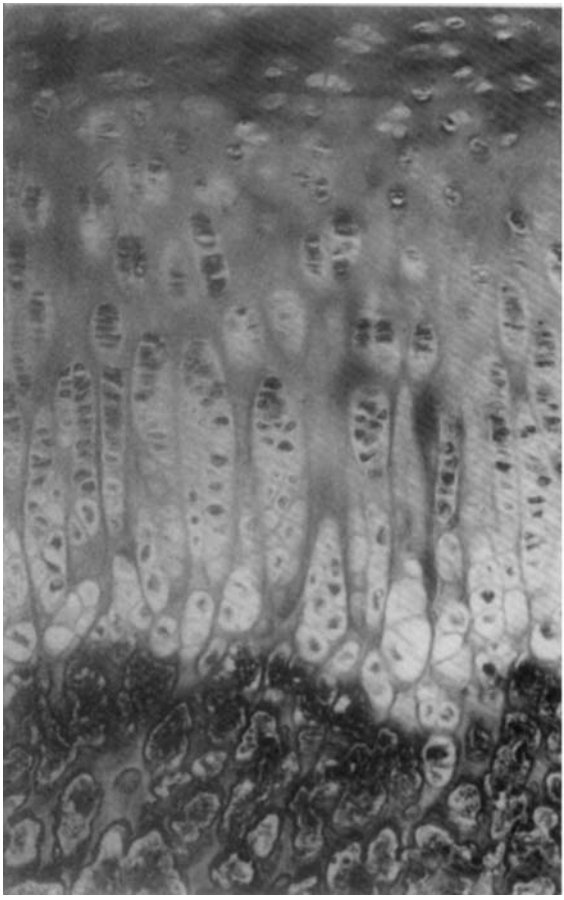


B. Growth plate, cross section. (Gomori, x 150)

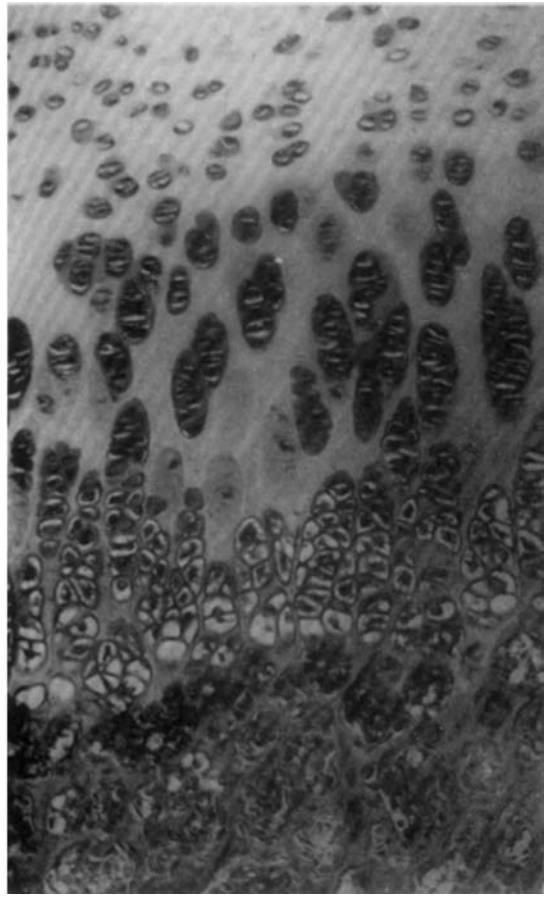
The cartilage above the growth plate appears to be homogeneous whereas reticulin fibrils are seen in the matrix between the cell columns.

C. Growth plate, cross section. (Alcian green, x 150)

The cells of the upper part of the growth plate are strongly Alcian-positive, whereas the most swollen cells are not stained.



B

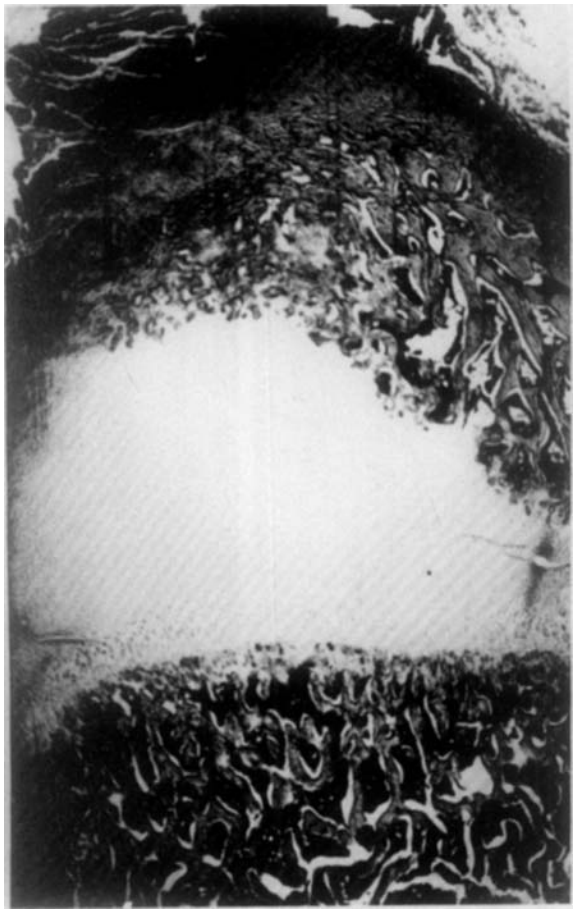


C

Figure 7. Histological sections from the posterior third of the iliac crest. Observation period: 12 weeks (7-month-old dog).

A. General view, cross section. (Haemalun-azophloxine-saffron, x 15)

A secondary centre of ossification is seen at the top.



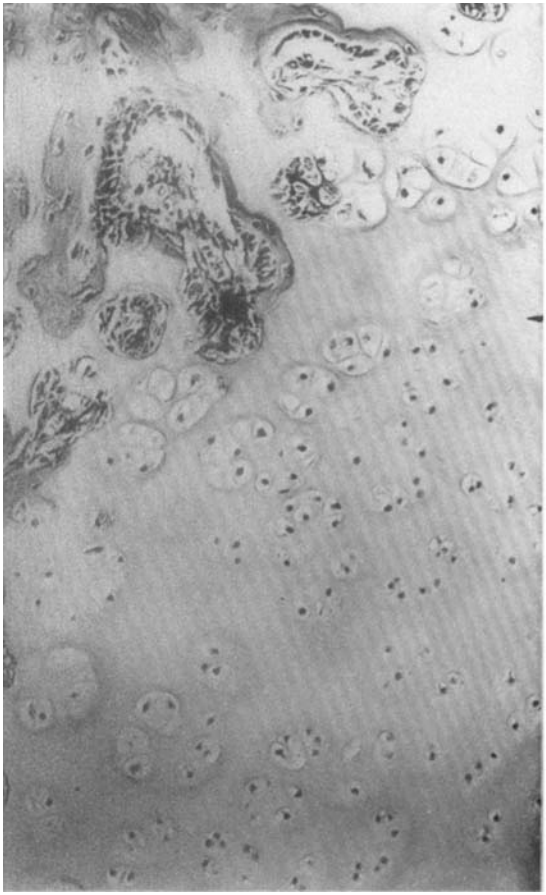
A

B. Detail from the ossification centre of Figure 7A. (Haemalun-azophloxine-saffron, x 150)

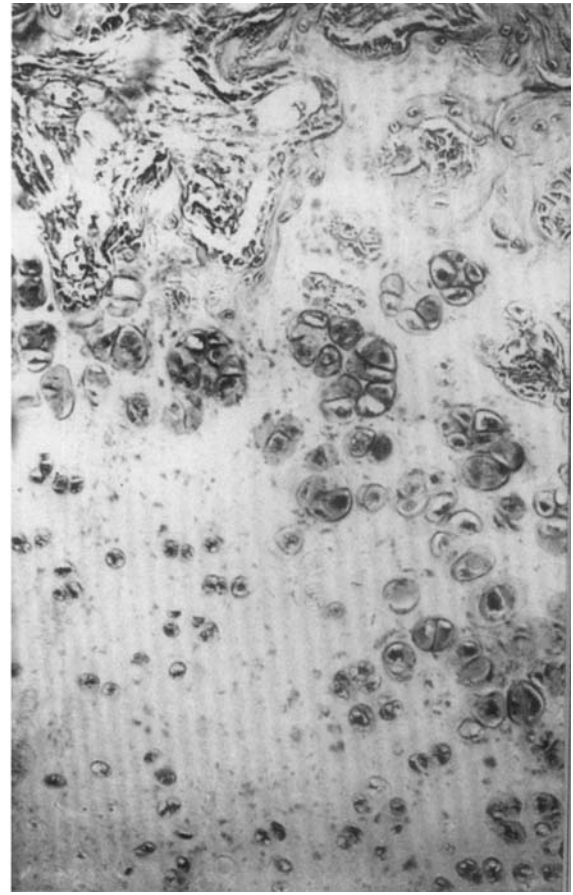
Rather large cartilage cells, partly arranged in clusters, are invaded by small vessels surrounded by connective tissue. Ossification of the cartilage matrix is seen around these vessels.

C. Detail from the ossification centre of Figure 7 A. (Alcian green, x 150)

Most of the moderately swollen cells are strongly Alcian-positive.



B



C

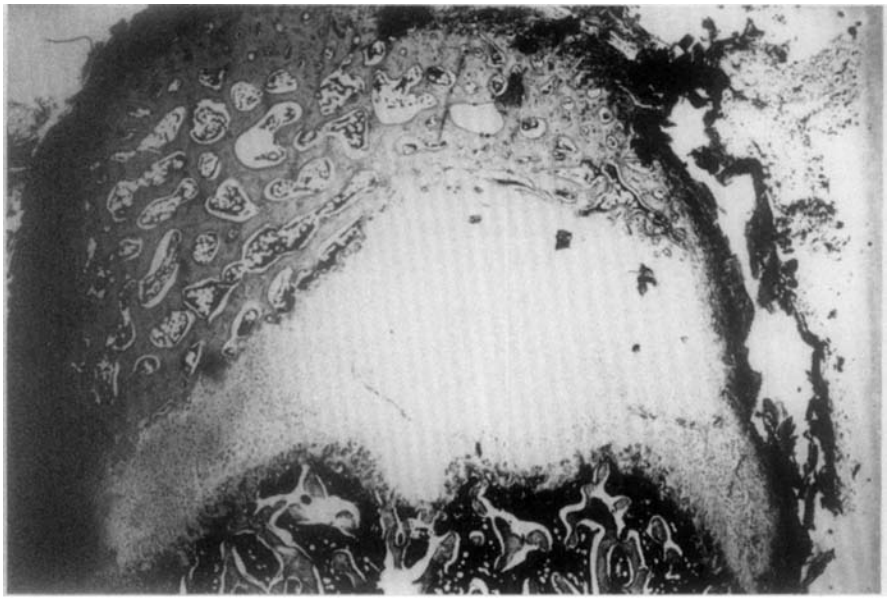
Figure 8. Histological sections from the posterior third of the iliac crest. Observation period 6 months (9½-month-old dog).

A. General view, cross section. (Haemalun-azophloxine-saffron, x 15)

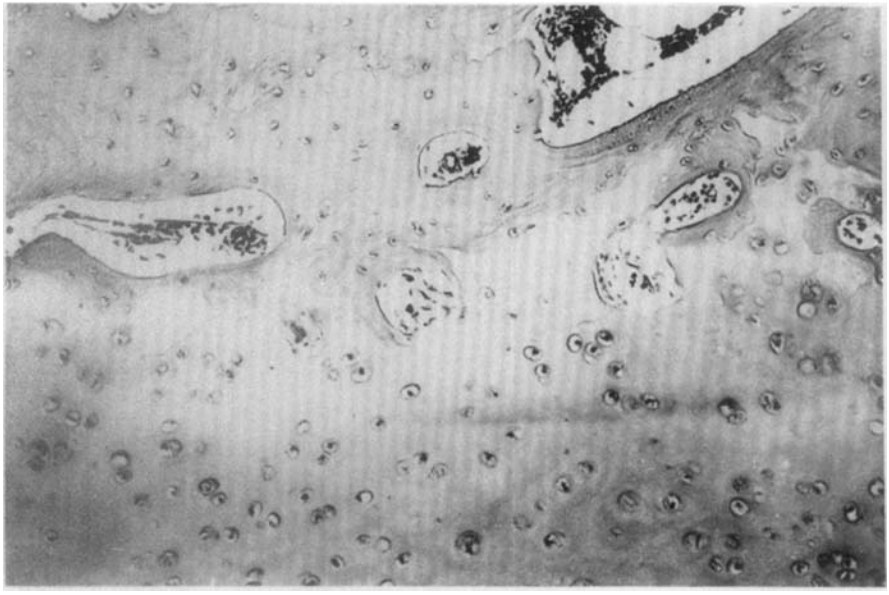
A great part of the apophysis has become bone by secondary ossification.

B. Detail from the secondary ossification centre of Figure 8 A. (Haemalun-azophloxine-saffron, x 150)

The cartilage cells near the ossification zone are small and not arranged in clusters.



A



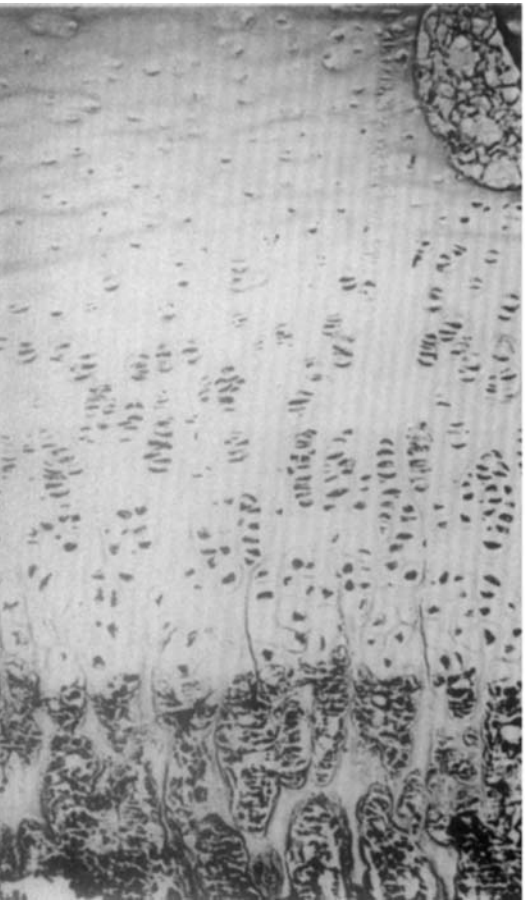
B

Figure 9. Histological sections from the iliac crest. The growth plate at various times of observation. (Haemalun-azophloxine-saffron, x 150)

The sections show that the height and the regularity of the cell columns of the growth plate decrease from 12 weeks to 9 months. At 9 months, however, there are still some columnar or cluster arrangements of swollen cartilage cells in the middle third of the iliac crest.

A. Observation period: 12 weeks (7-month-old dog). From the middle third, cross section.

B. Observation period: 6 months (9½-month-old dog). From the middle third, cross section.

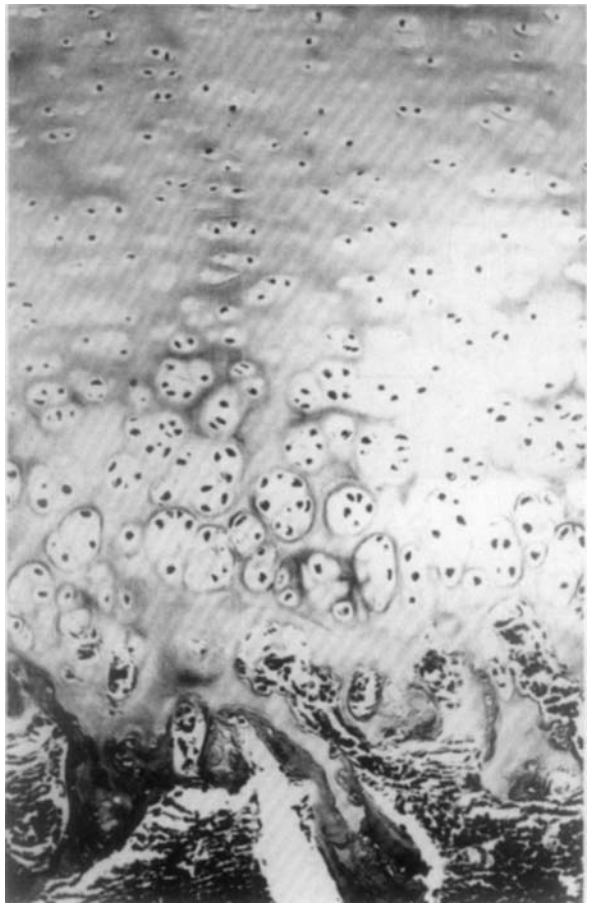


A

B

Figure 9. Continued)

C. Observation period: 9 months (12½-month-old dog). From the middle third, cross section.



D. Observation period: 9 months (12½-month-old dog).

From the posterior third, cross section.
(From the same animal as Figure 9 C).

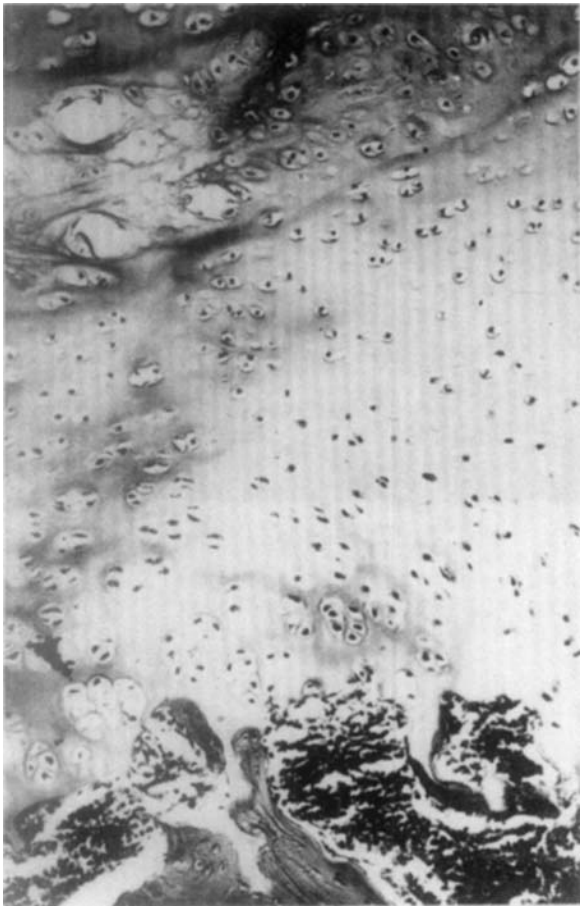
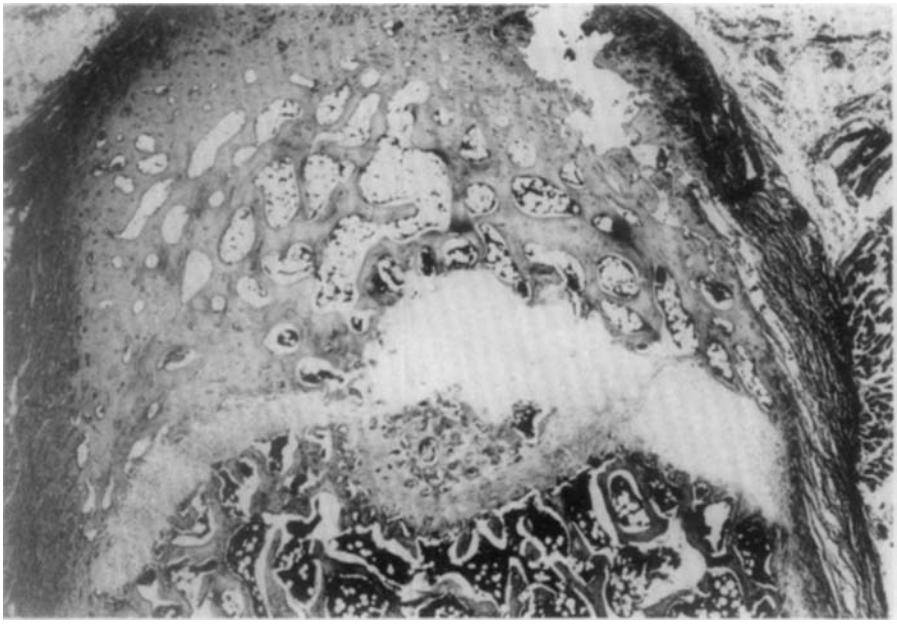


Figure 10. Histological sections from the iliac crest. Completion of the ossification.

A. Observation period: 9 months (12-month-old dog)

From the posterior third, cross section. (Haemalun-azophloxine-saffron, x 15)

Only a narrow zone of cartilage remains unossified in the basal part of the apophysis.



B. Detail from Figure 10 A. (x 90)
There are no signs of proliferative activity of the cartilage cells. Swollen cartilage cells are seen neither at the apophyseal side (above) nor at the metaphyseal side (below).

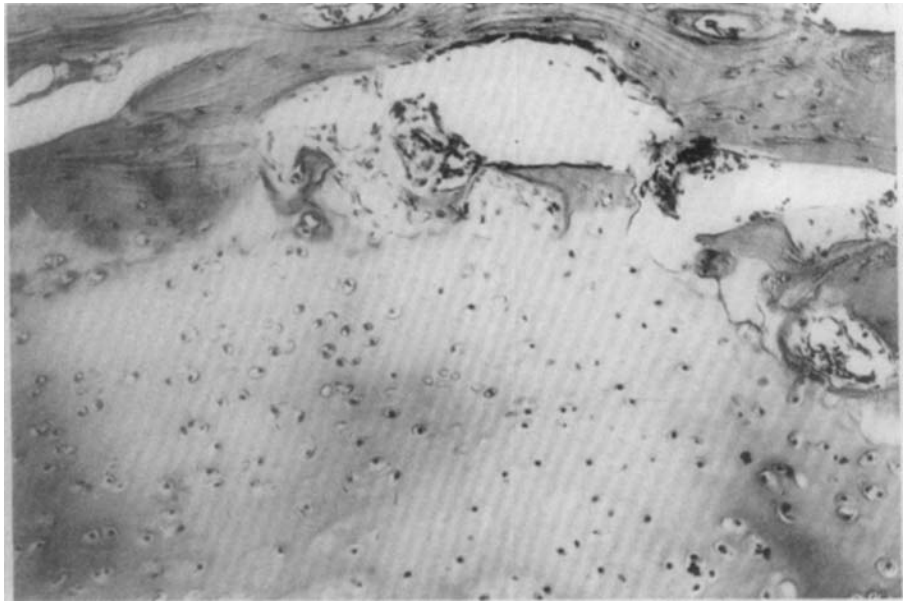


Figure 10. (Continued)

C. Observation period: 14 months (18-month-old dog). (Haemalun-azophloxine-saffron, x 15)

From the middle third, cross section.
The ossification is completed.

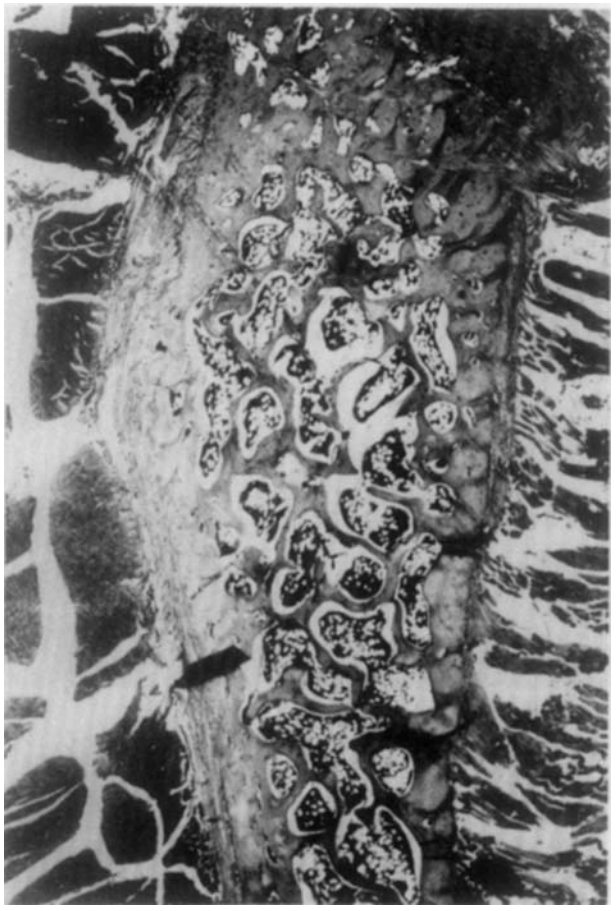


Figure 11. Macroscopical appearance of the operated joints at various periods of observation.
(In all the joints the osteochondral transplant - transplant A - has been placed in the joint surface of the right condyle whereas the control has been implanted into the left.)

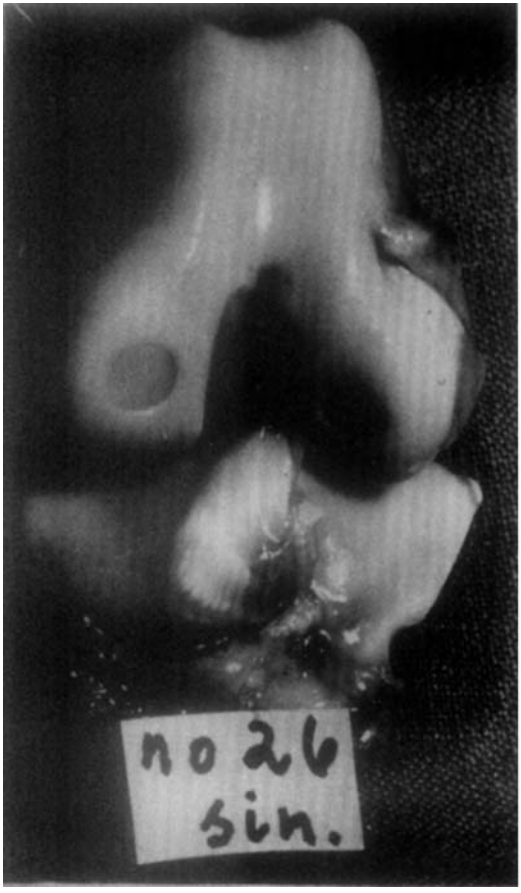
A. Observation period: 2 weeks

Osteochondral transplant A.: The cartilage is smooth and shining, the surface is slightly depressed.
Control-osseous transplant: The defect is clearly visible, its area seems to be unchanged.

B. Observation period: 6 weeks

Osteochondral transplant A.: The surface is still smooth and shining, slightly depressed. Medially a cleft filled with greyish tissue is seen.
Control-osseous transplant: The area of the defect has been strongly reduced by ingrowth of cartilage anteriorally and posteriorally. This cartilage seems to originate from the surrounding joint cartilage. The surface corresponding to the original defect is slightly depressed.

A



B

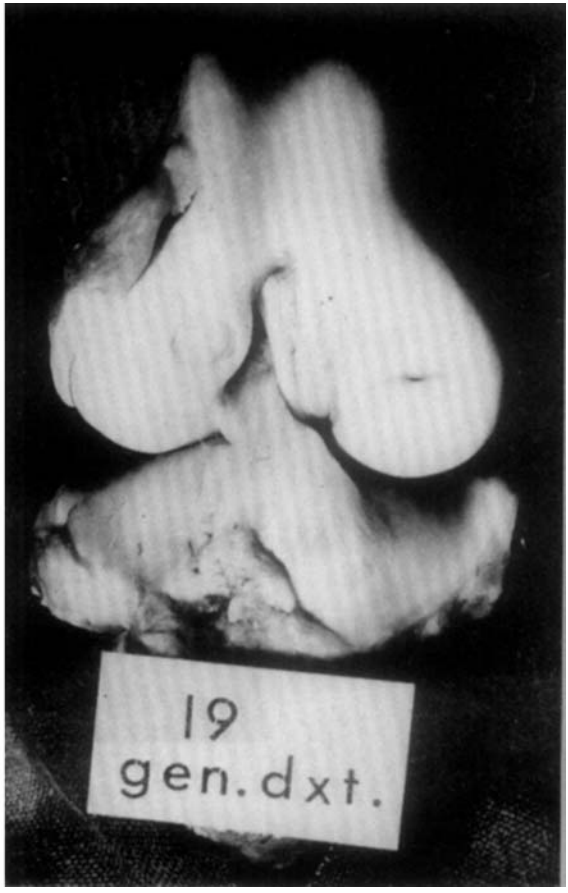
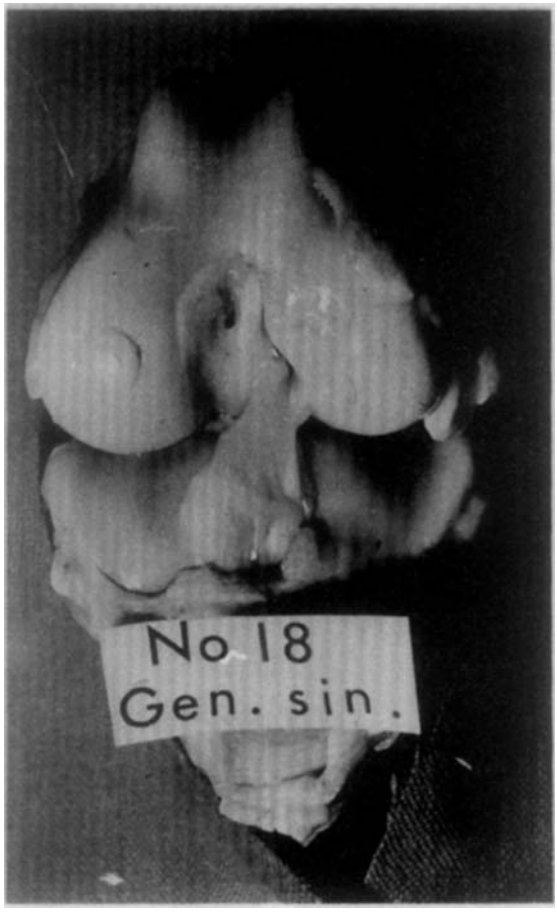


Figure 11. (Continued)

C. Observation period: 12 weeks

Osteochondral transplant A.: The surface is still smooth and shining and obviously cartilaginous, medially the surface of the transplant is level with the joint cartilage, laterally there is an elevation of the transplant. Control-osseous transplant and devitalized cartilage: There persists only a small circular defect centrally, the peripheral part of the defect has been covered with cartilage which apparently has been created by ingrowth of the surrounding joint cartilage. Also centrally a cartilaginous tissue is seen. The whole surface corresponding to the original defect is markedly depressed.



D. Observation period: 9 months

Osteochondral transplant A.: The cartilage of the transplant is smooth and shining and completely level with the surrounding joint surface. The border to the surrounding cartilage is still visible.
Control-osseous transplant and devitalized cartilage: As seen in Figure 11 C, except that the surface corresponding to the original defect is less depressed.

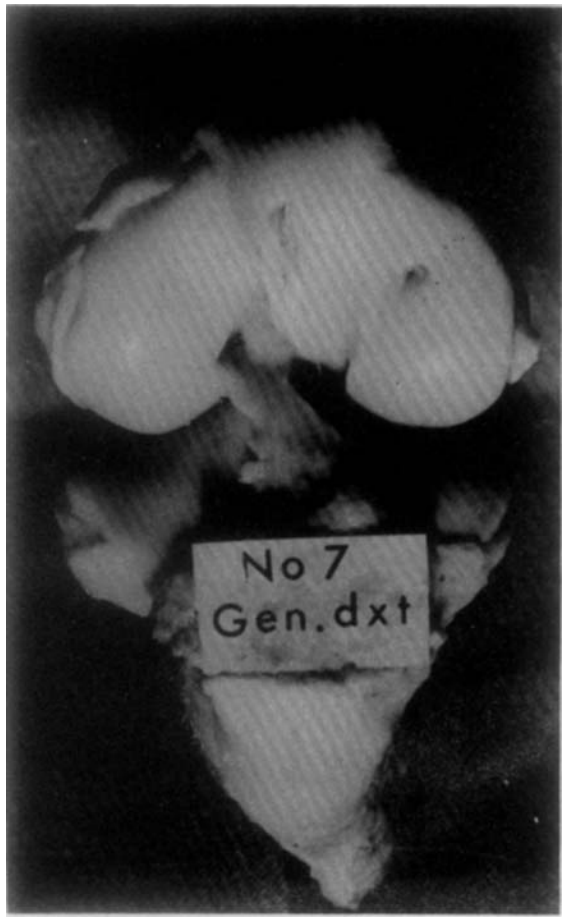


Figure 11. (Continued)

E. Observation period: 14 months

Osteochondral transplant A: The surface of the transplant appears still to be cartilaginous. A slight protrusion is seen.
Control-osseous transplant: The area of the defect has been slightly reduced - apparently by ingrowth of the surrounding joint cartilage. Some cartilaginous tissue is seen centrally.

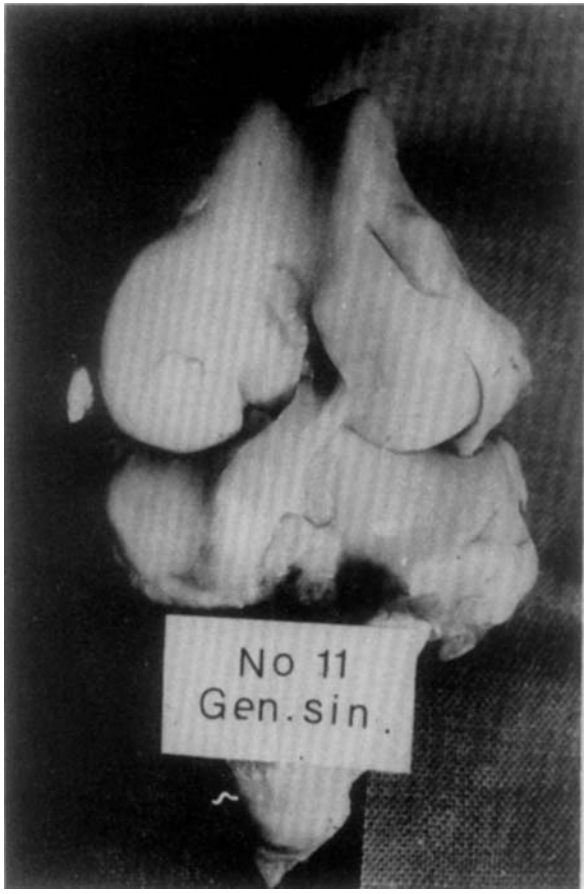


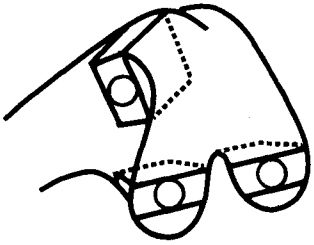
Figure 12.

A. Removal of transplants for histological examination.

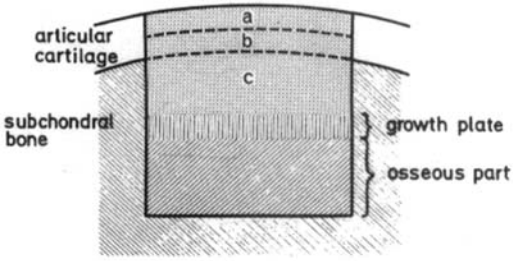
The transplants within the joint surface were principally removed by transversal resections (exceptions: Se the text). The transplants outside the joint surface were removed by resections at right angle to the length axis of the femur.

B. The different zones of the transplants.

For explanation: See the text (p. 22).



A



B

Figure 13. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Observation period: 2 weeks.

A. Vital cells (from zone c) Haemalun-azophloxine-saffron, x 580)
The cells have large, basophilic nuclei.
There is a clearly visible cytoplasm.

B. Non-vital cells (from zone c) (Haemalun-azophloxine-saffron, x 580)
The cells contain pycnotic and peripherally located nuclei; there is no staining of the cytoplasm.

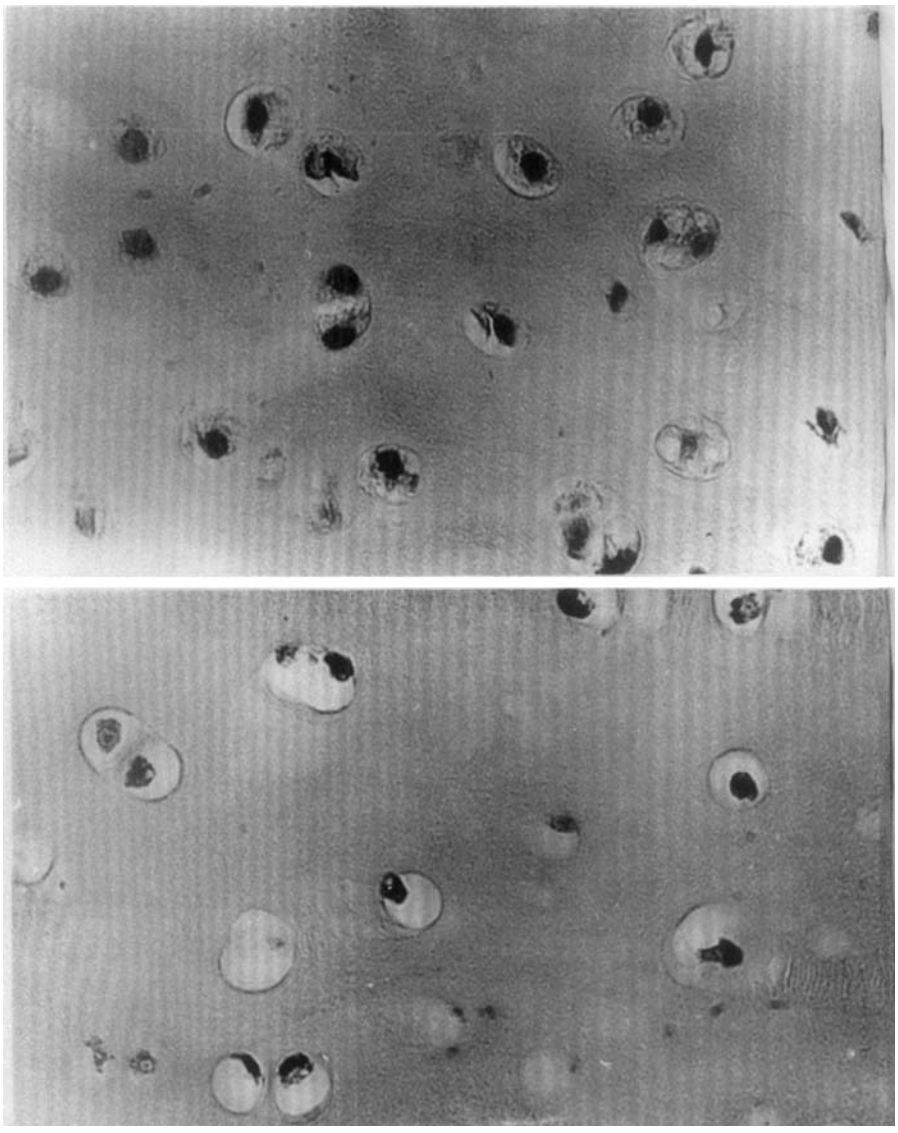


Figure 14. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Observation period: 2 weeks.

A. From zone c and the upper part of the growth plate.
(Haemalun-azophloxine-saffron, x 40)
The picture shows a transplant where the greatest part of the growth plate and the central part of the overlying zone c have become necrotic. The whole of the growth plate is necrotic, except the cells of the superior part of some peripherally located cell columns (at the left side). The pale area, which is seen centrally and which becomes narrower towards zone b, represents the necrotic part of zone c. In the basal region small vessels and connective tissue penetrate into the necrotic area.

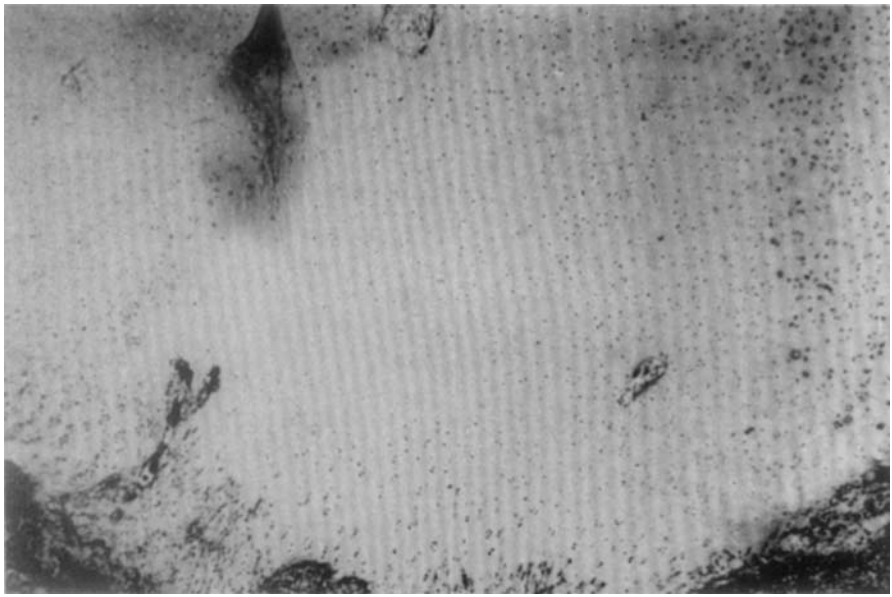
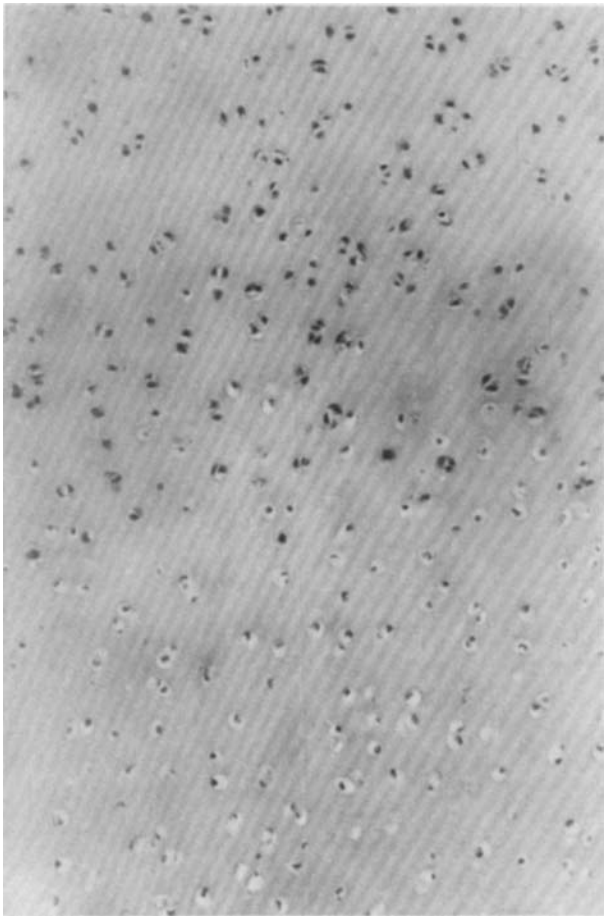


Figure 14. (Continued)

B. Detail from Figure 14 A. (Haemalun-azophloxine-saffron, x 150)
The picture shows the transition between vital cartilage (upwards) and necrotic cartilage.



C. From same region as shown in Figure 14 B.
(Alcian green, x 150)
The cytoplasm of the non-vital cells is, in contrast to that of the vital cells, Alcian-negative.

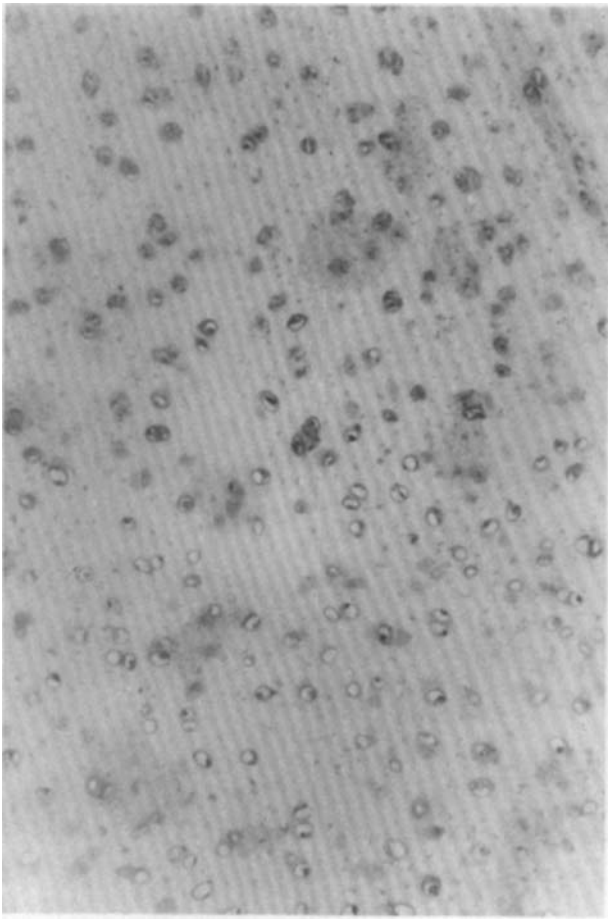
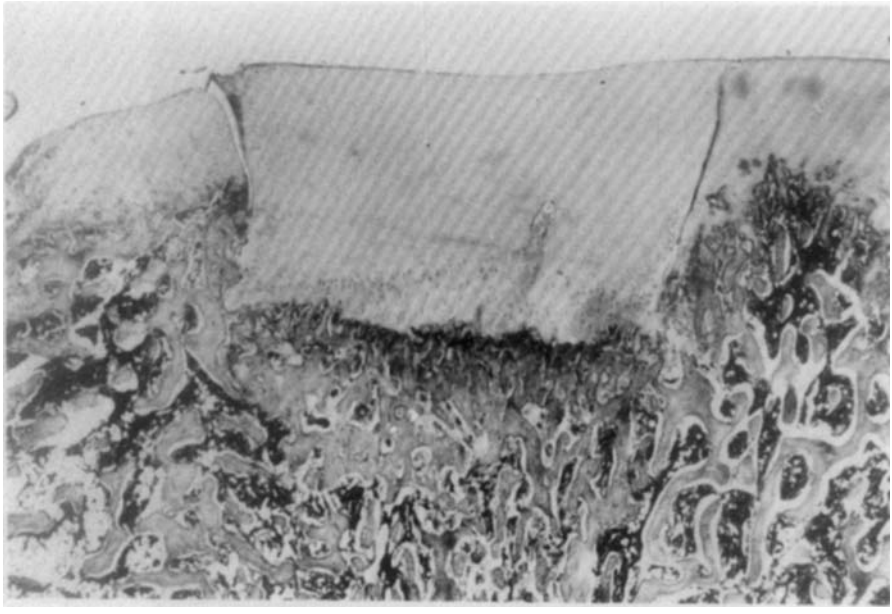


Figure 15. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A). Observation period: 2 weeks.

A. General view. (Haemalun-azophloxine-saffron, x 15)

The pale area in the middle part of the growth plate represents a necrotic region (see Figure 15 C). There is a close contact between the transplanted cartilage and the joint cartilage but no signs of union. In the osseous part of the transplant there is an almost complete lack of blood vessels and fat cells in the marrow spaces.



B. From the middle part of the growth plate of the transplant shown in Figure 15 A.
(Haemalun-axophloxine-saffron, x 40)

The picture shows that the central part of the growth plate is regenerated from the cells overlying the necrotic columnar zone. The interspaces of the subchondral bone are filled with connective tissue.

Figure 15. (Continued)

C. Detail from the growth plate shown in Figure 15 B.
(Haemalun-azophloxine-saffron, x 150)

Above: Intensive proliferation of cells.
Below: Columns of cells with pycnotic nuclei.

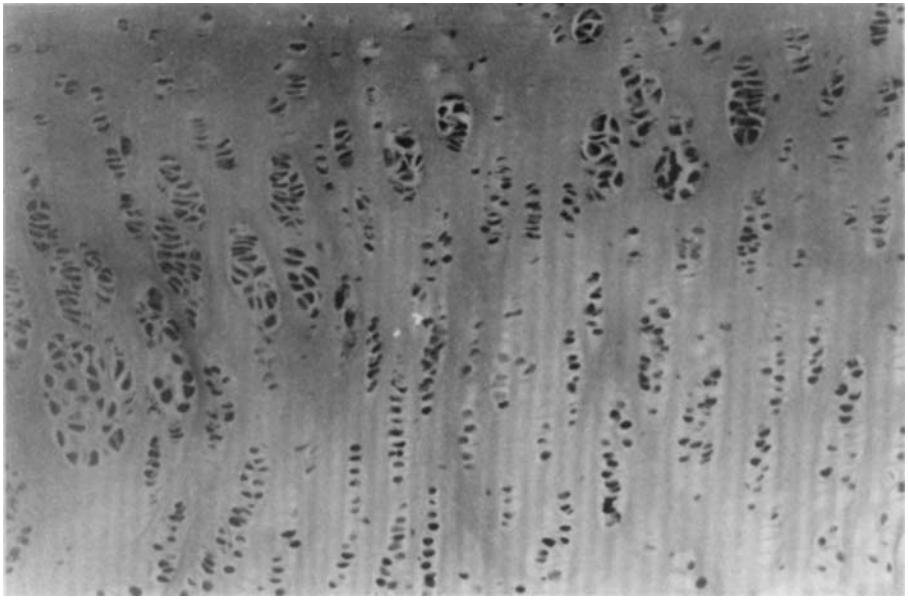


Figure 16. Extension of the necrotic areas within the osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Observation period: 2 weeks.

The upper curved line in the middle part of the transplant indicates the demarcation of the maximal necrosis. The lower curved line indicates the demarcation of the minimal necrotic area.

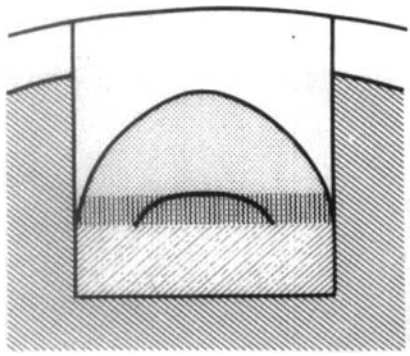
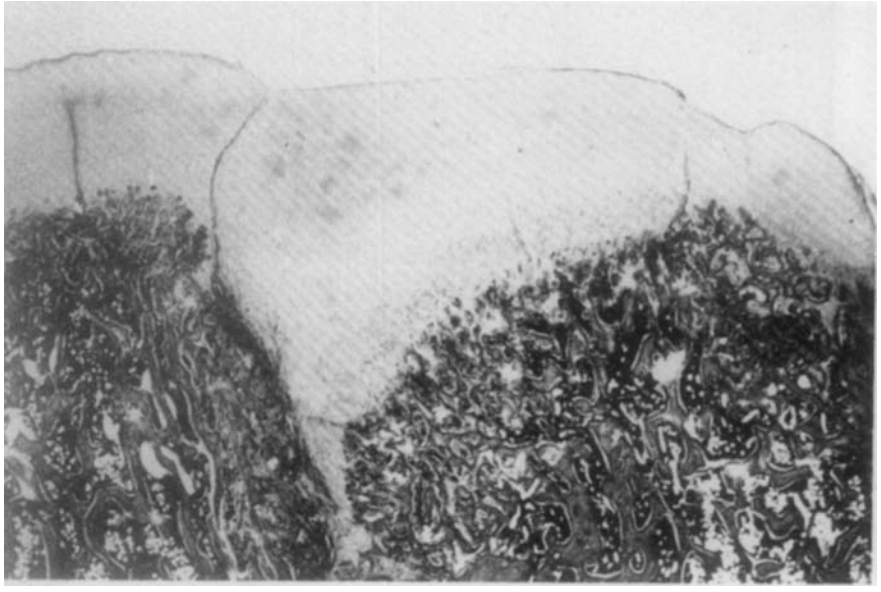


Figure 17. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Observation period: 6 weeks.

A. General view of a transplant.
(Haemalun-azophloxine-saffron, x 15)

To the right the cartilaginous part of the transplant has become ossified almost completely out to the level of the base of the surrounding joint cartilage. In the left half of the transplant islets of cartilage tissue are seen within the subchondral bone tissue. (The cells of these islets appeared to be necrotic when examined under higher magnification.) To the left there is a close contact between the transplanted cartilage and the joint cartilage but no signs of union. To the right an interspace of about 0.5 mm width is seen and this is bridged by a cartilage-like tissue. There is no visible cleft between the transplant and the bone bed in zone c.

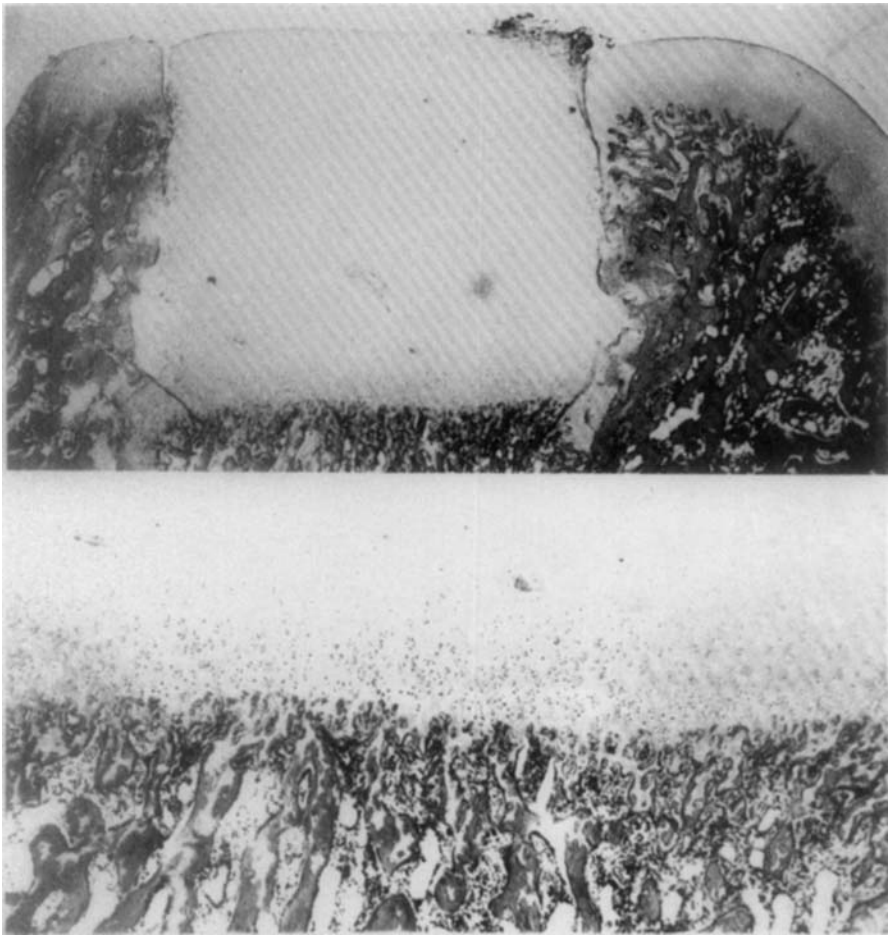


B. General view of a transplant.
(Haemalun-azophloxine-saffron, x 15)

In the subchondral bone tissue islets of cartilage are seen (compare to Figure 17 C). Only the basal part of the transplanted cartilage has become ossified. On both sides of the transplant there is a very close contact between the transplanted cartilage and the joint cartilage but no signs of union. The surface of the transplant is level with that of the surrounding part of the joint.

C. Detail from Figure 17 B.
(Haemalun-azophloxine-saffron, x 40)

The cartilage islets of the subchondral bone contain necrotic cartilage cells. Down to the left scanty remnants of a necrotic growth plate are seen; the osseous region above these represents bone tissue formed by ossification of the transplanted cartilage. Ossification is seen in the basal part of the regenerated growth plate.

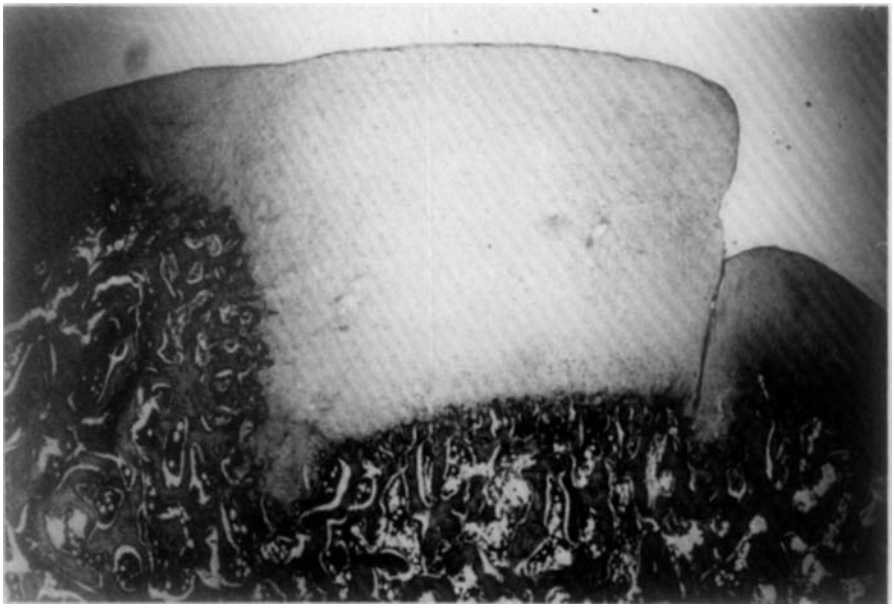


C

Figure 18. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Observation period: 12 weeks.

A. General view. (Haemalun-azophloxine-saffron, x 15)

To the right the transplant has become ossified almost completely out to the level of the base of the adjacent joint cartilage. On the same side there is a very close contact between the transplanted cartilage and the joint cartilage but no signs of union:, further, the surface of the transplant is considerably elevated. On the left side the transplanted cartilage has united to the joint cartilage (for detail: Figure 25 C) and the surface of the transplant is here level with that of the joint cartilage. The transplant is connected to the bone bed by bone tissue.



B. Detail from the basal part of the transplant shown in Figure 18 A. (Haemalun-azophloxine-saffron, x 150)

The growth plate contains low and highly irregular columns of cells or clustered cells. The cells at the bottom of the columns are markedly swollen. Enchondral ossification takes place.

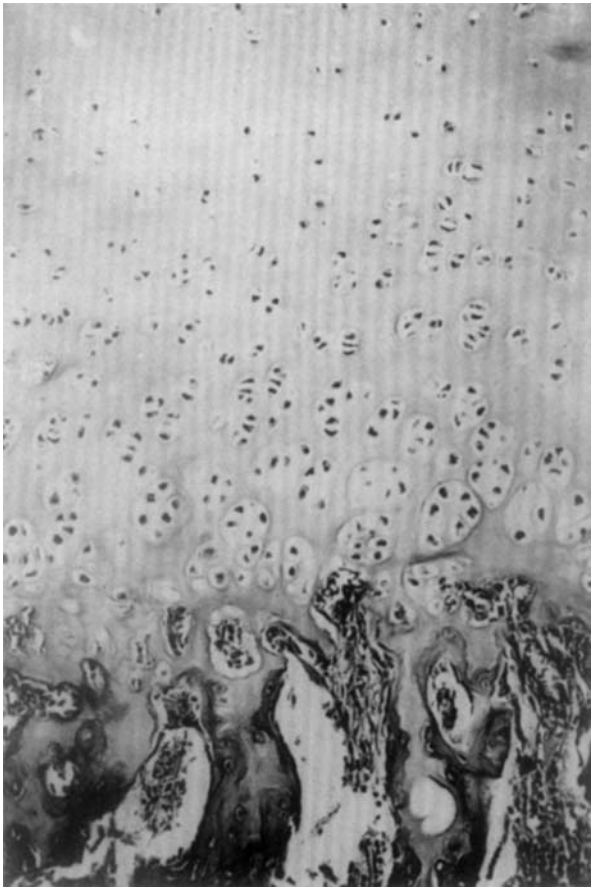
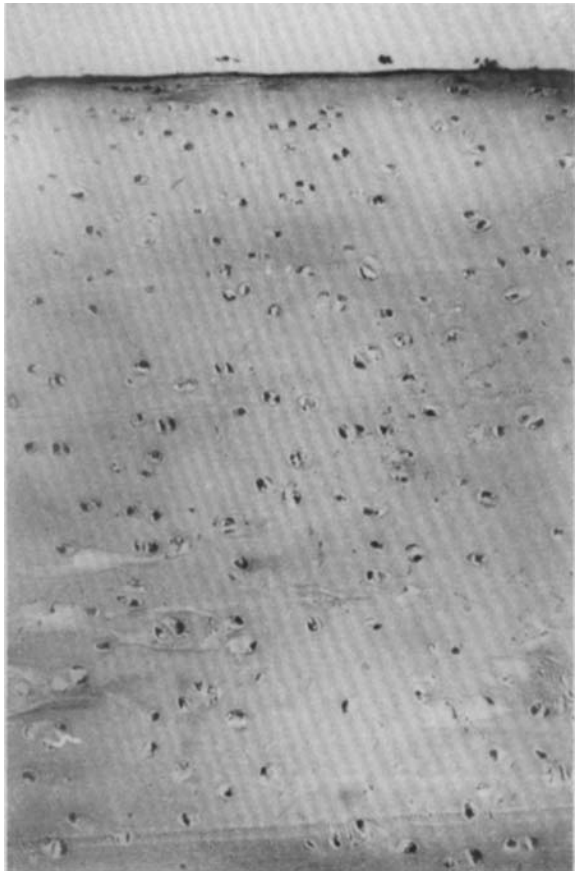


Figure 19. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Observation period: 12 weeks.

A. From the superficial zone a.
(Haemalun-azophloxine-saffron, x 150)

The cells are vital. The most superficial cells are slightly flattened and they are orientated parallel to the surface of the transplant. A few double cells are seen.



B. From the same region as shown in Figure 19 A.
(Alcian green, x 150)

Cell nuclei, cytoplasm and matrix of the cartilage are Alcian-positive.

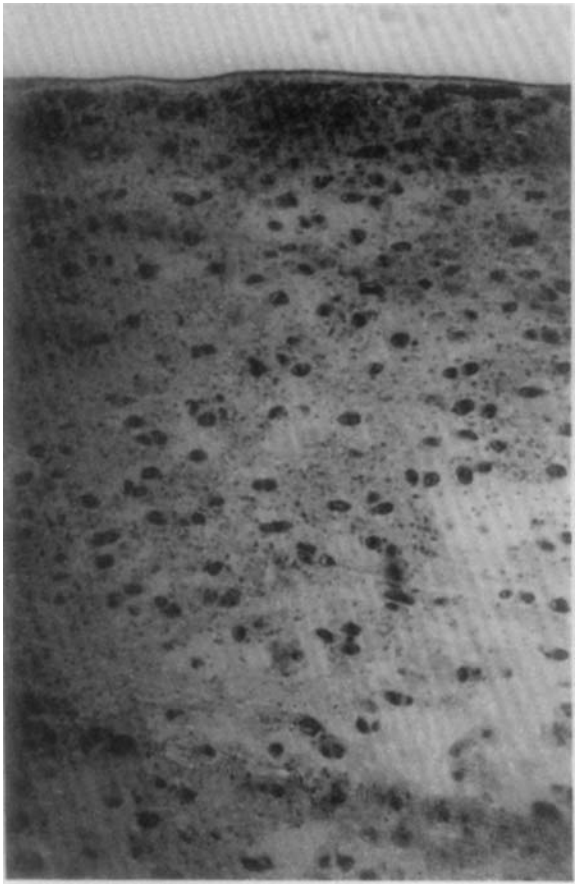
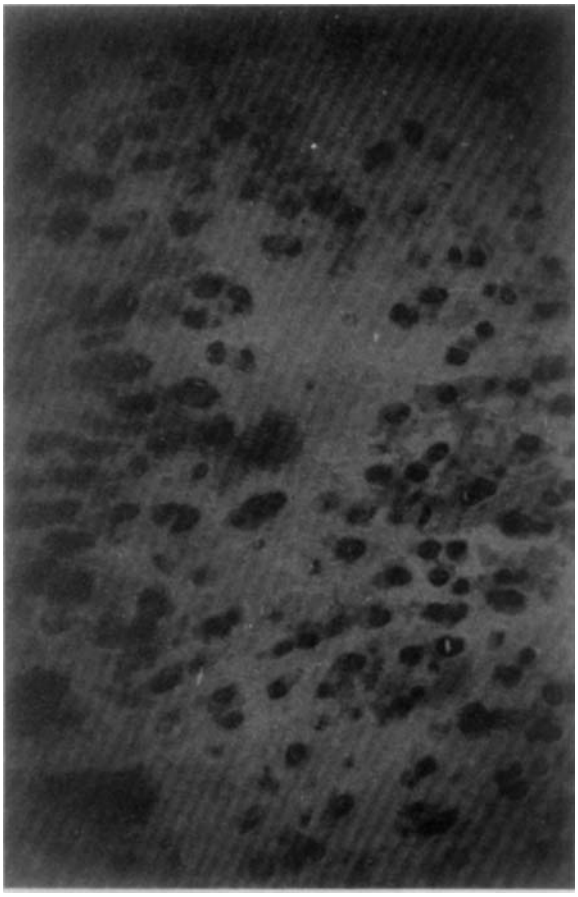


Figure 19. (Continued)

C. From zone c.

(Haemalun-azophloxine-saffron, x 150)

The cells of this zone are somewhat larger than the cells of the superficial zone a. In this zone the cells are also vital.



D. From the same region as shown in Figure 19 C.
(Alcian green, x 150)

The cells are rich in Alcian-positive cytoplasm, cell nuclei and matrix are also Alcian-positive.

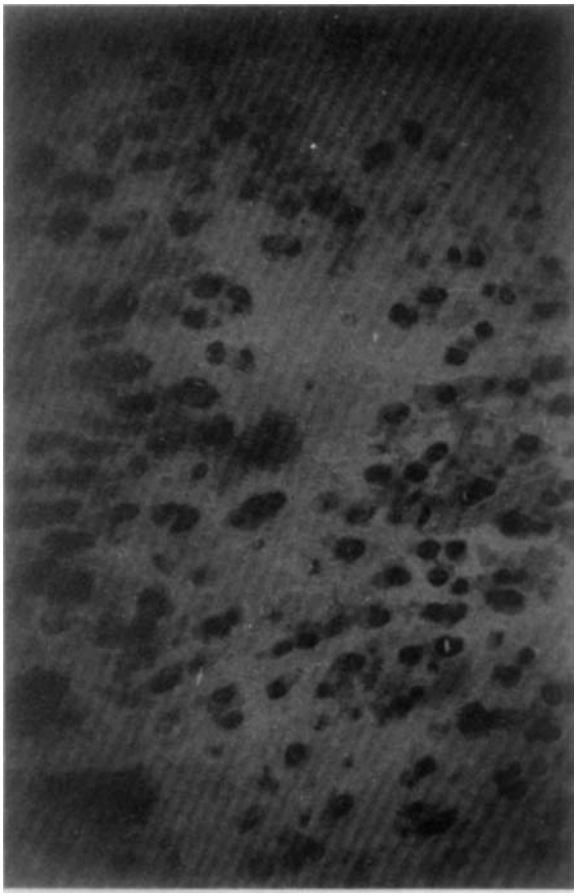
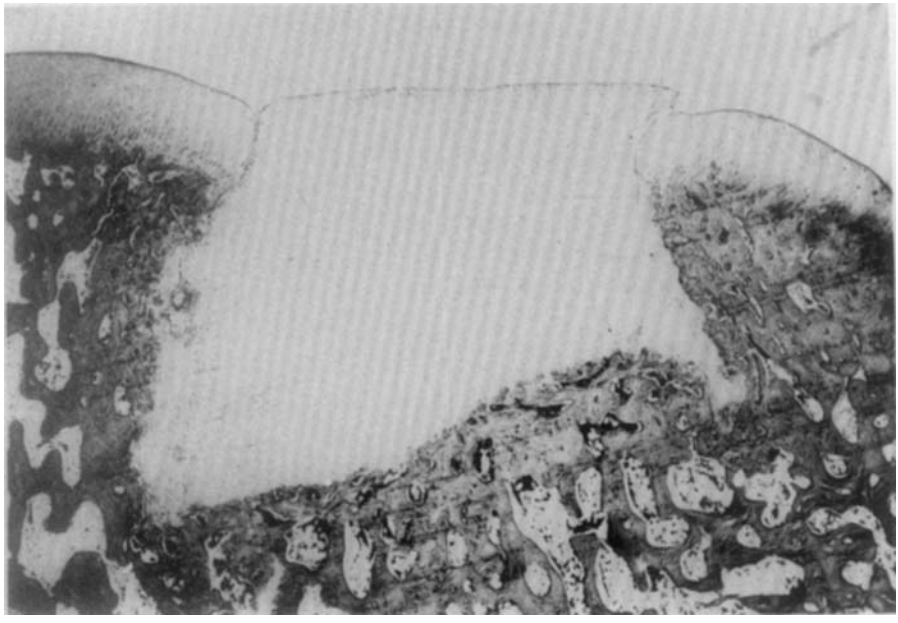


Figure 20. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Observation period: 6 months.

A. General view.
(Haemalun-azophloxine-saffron, x 15)

The ossification of the transplanted cartilage has not reached the superficial zones a and b. On both sides of the transplant there is a close contact between the transplanted cartilage and the joint cartilage but no union. On the right side there is a slight protrusion of the transplant. In zone c the cartilage has united to the surrounding bone tissue.



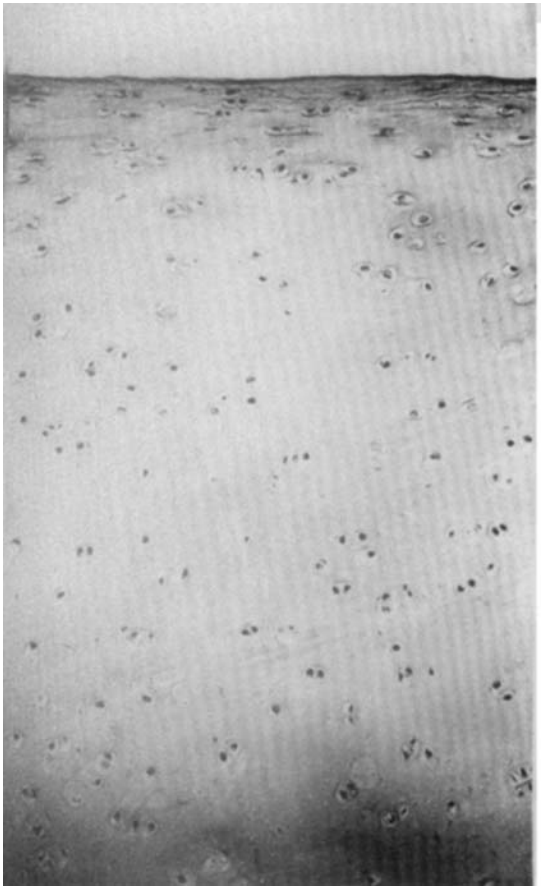
B. From the superficial zone a.
(Haemalun-azophloxine-saffron, x 150)

The cells are vital; superficially the cells are more clearly orientated parallel to the surface of the transplant than at the previous observations.

C. From zone c.
(Haemalun-azophloxine-saffron, x 150)

The cells are slightly swollen. In the basal region ossification is seen even without the existence of any growth plate.

B



C

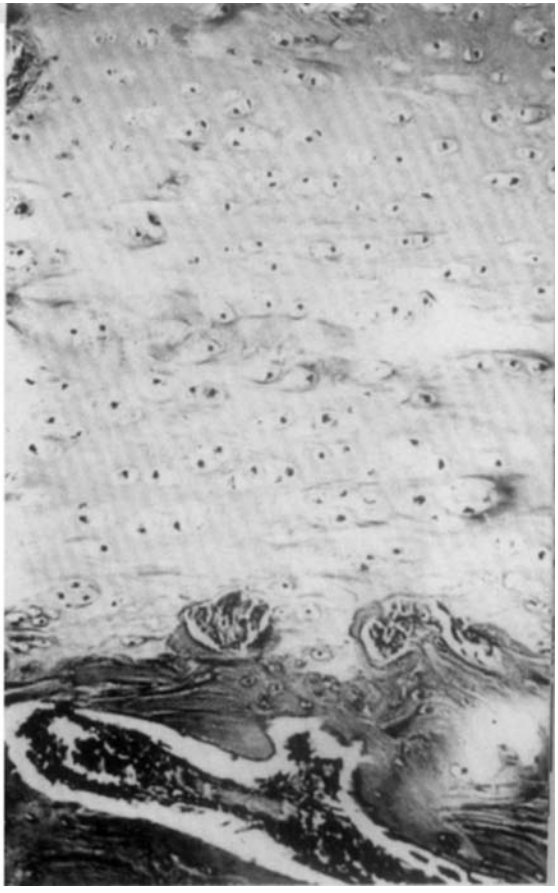
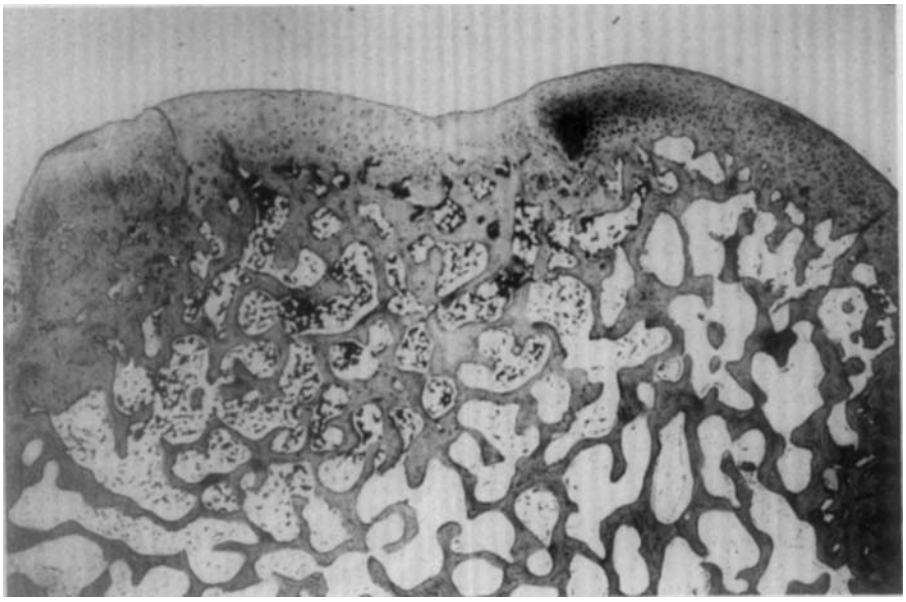


Figure 21. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Observation period: 9 months.

A. General view. (Haemalun-azophloxin-saffron, x 15)

The transplanted cartilage has become completely ossified right out to the basal level of the surrounding joint cartilage. There is no ossification in zone a or b.
To the left there is a close contact between the transplanted cartilage and the joint cartilage but no union has taken place. To the right the transplanted cartilage is connected to the joint cartilage. (By examination under greater magnification it was found that there was a bridge of fibrocartilage of 0.5 mm width on the right side). On the left side the surface of the transplant is level with the joint cartilage, on the right side there is a slight depression corresponding to the fribrocartilage. The trabecular structure of the subchondral bone of the transplant does not differ significantly from that of the rest of the condyle.



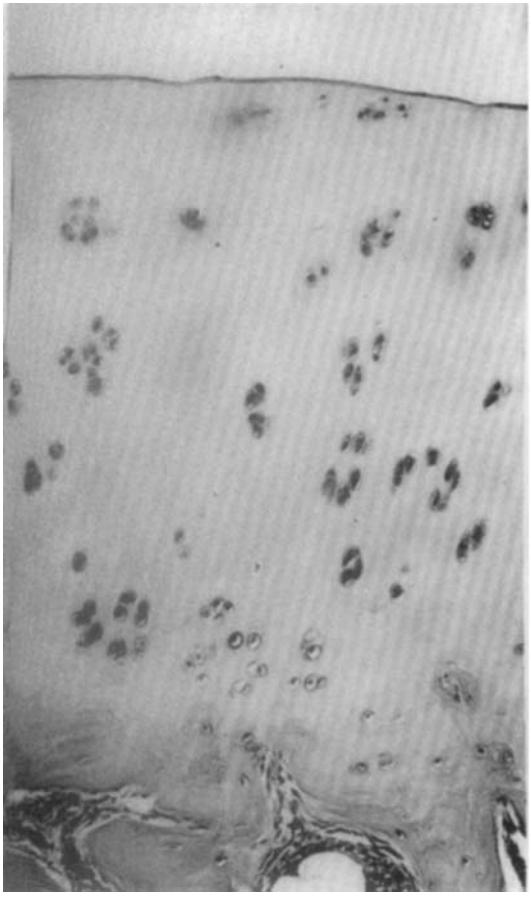
B. From the superficial zones a and b of the transplant shown in Figure 21 A. (Haemalun-azophloxine-saffron, x 150)

The cartilage cells are vital. In the basal part of the cartilage a zone resembling the basal calcified zone of normal joint cartilage is seen (compare Figure 21 C).

C. Joint cartilage from the same section.
(Haemalun-azophloxine-saffron, x 150)

The cartilage contains relatively more cells than the transplanted cartilage. A slightly basophilic zone is seen in the basal part. In this zone the cells are pale.

B



C

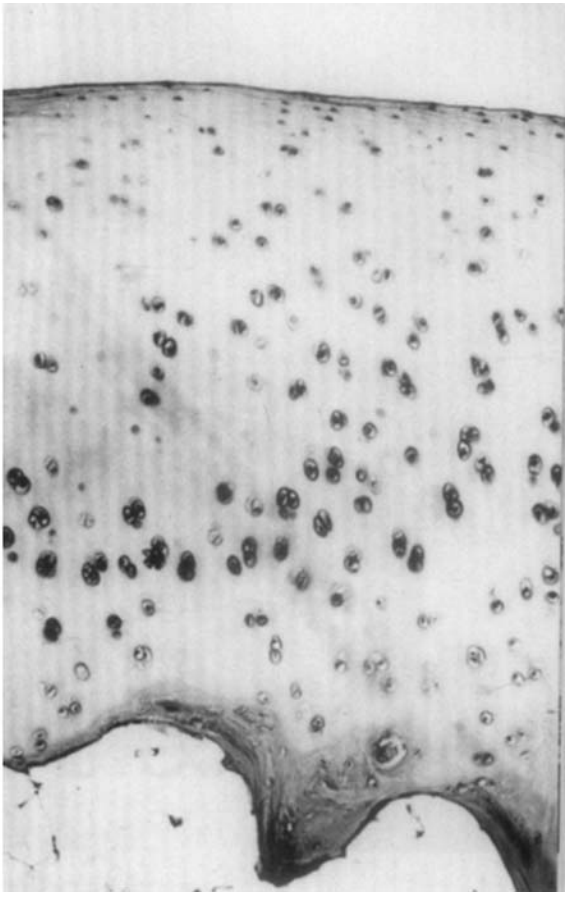
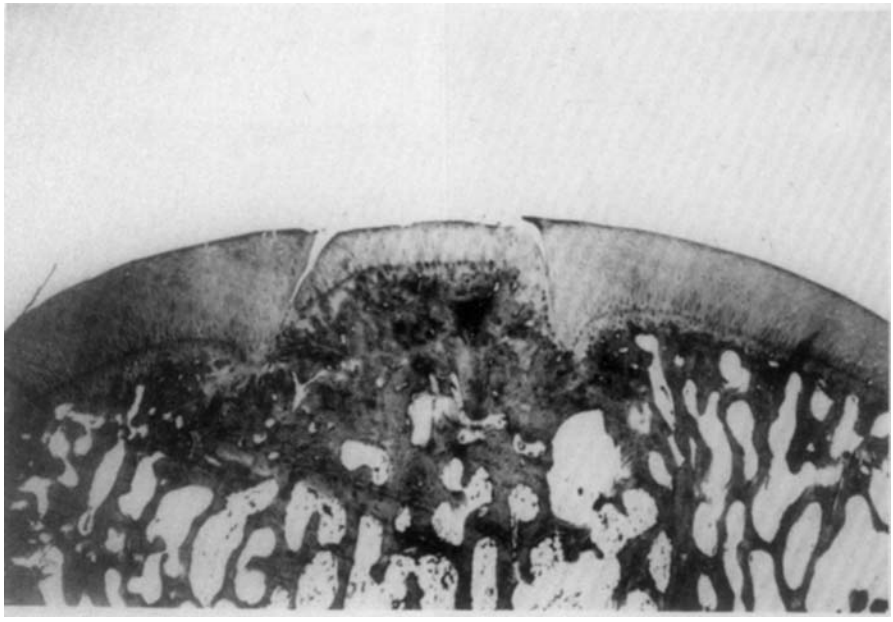


Figure 22. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Observation period: 14 months

A. General view of a transplant.
(Haemalun-azophloxine-saffron, x 15)

The surface of this transplant is level with the surrounding joint surface and there are no signs of destruction of the cartilage. There is a narrow cleft between the transplanted cartilage and the joint cartilage and no signs of union.
Note the clearly demarcated calcified zone in the basal part of the transplant.



B. Detail from the transplant shown in Figure 22 A.
(Haemalun-azophloxine-saffron, x 150)

The cells of the persisting cartilage are vital. Note the strong resemblance between the calcified basal zone in the transplanted cartilage and in the joint cartilage (compare Figure 22 C).

C. Detail from the joint cartilage.
(Haemalun-azophloxine-saffron, x 150)

For comparison to Figure 22 B.

B

C

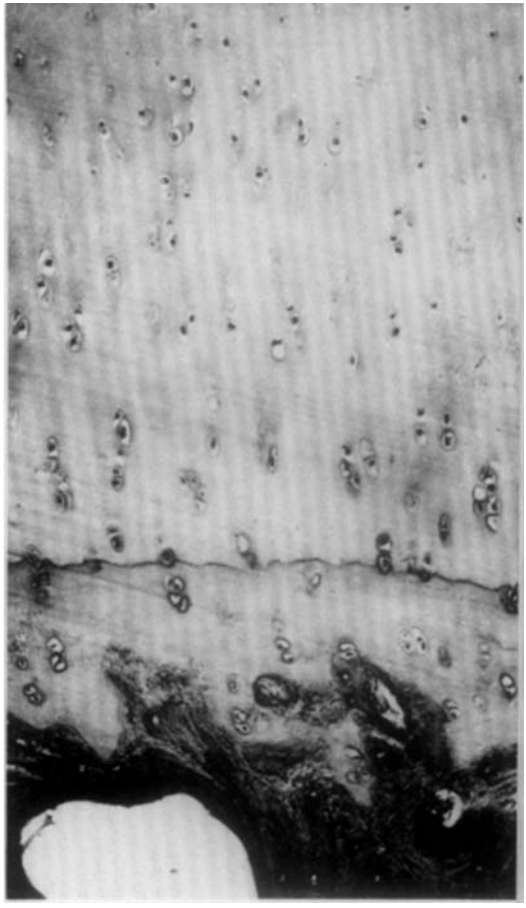


Figure 23. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Observation period: 14 months.

A. General view of a transplant.
(Haemalun-azophloxine-saffron, x 15)

As in the transplant shown in Figure 22 A the persisting unossified zone of cartilage is lower than the joint cartilage. To the right the transplanted cartilage has healed to the joint cartilage.
(Examinations at larger magnifications revealed that there was a 0.4-0.5 mm wide area filled with fibrocartilage at the junction between the transplanted cartilage and the joint cartilage). To the left there is a close contact, but no union.

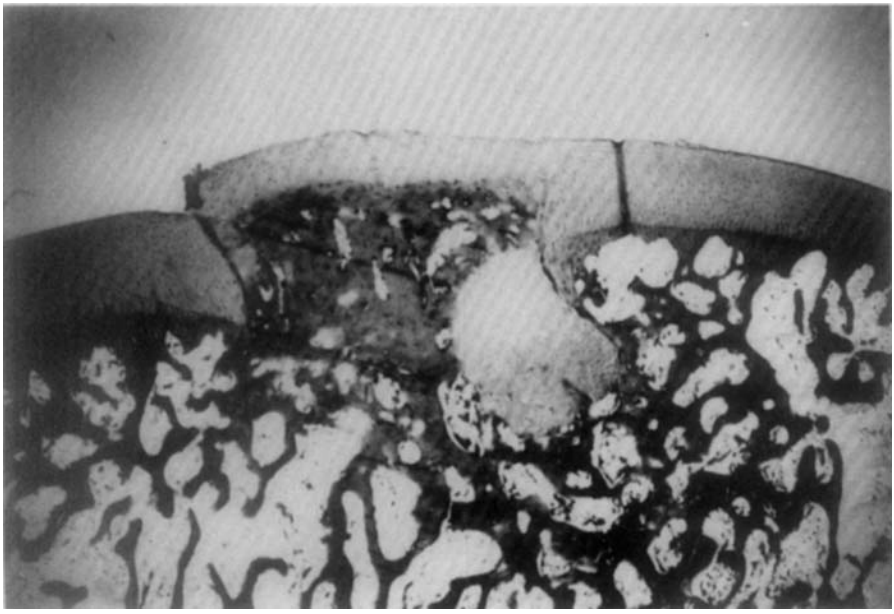
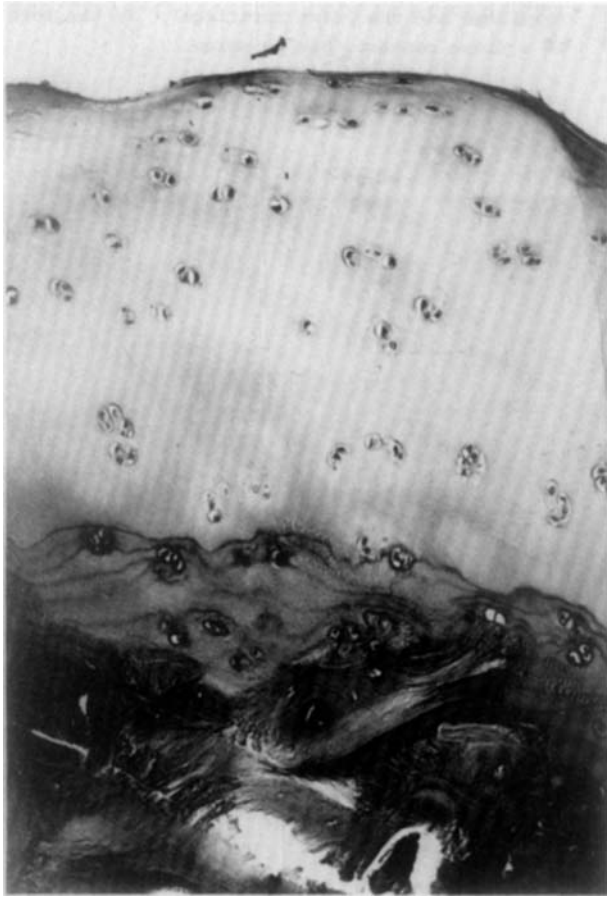


Figure 23. (Continued)

B. Detail from the transplant shown in Figure 23 A. (Haemalun-azophloxine-saffron, x 150)

In this transplant there is also a clearly demarcated calcified zone (compare Figure 22 B) which is similar to the calcified zone of the joint cartilage (compare Figure 23 C). The cells appear to be vital.



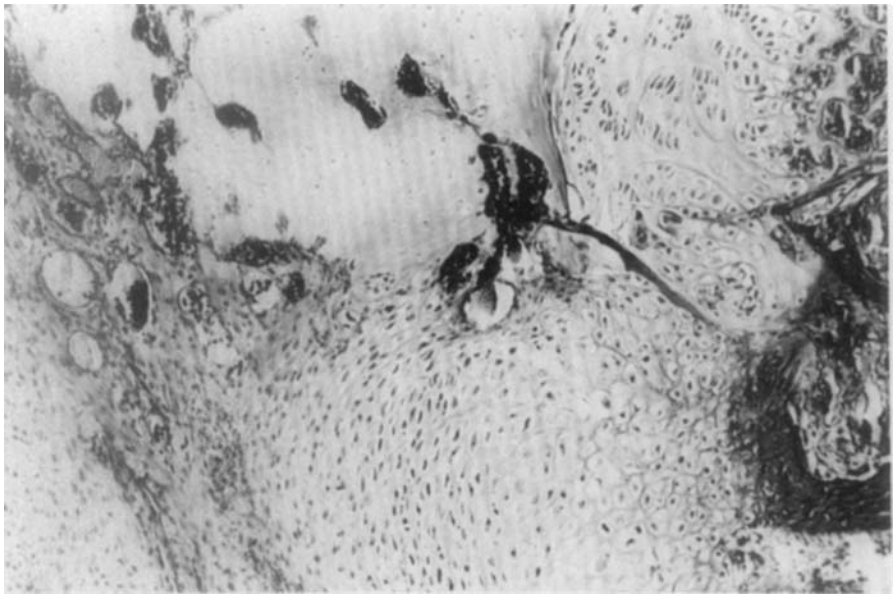
C. Detail from the joint cartilage adjacent to the transplant shown in Figure 23 A and B.
(Haemalun-axophloxine-saffron, x 150)

For comparison with Figure 23 B.

Figure 24. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A). Changes within necrotic cartilage.

A. From the growth plate/zone c. Observation period: 6 weeks.
(Haemalun-azophloxine-saffron, x 90)

To the left ingrowth of vessels and connective tissue is seen within necrotic cartilage and further metaplasia of connective tissue cells to cartilage cells. To the right the cartilage cells are markedly swollen and the cartilage undergoes ossification.



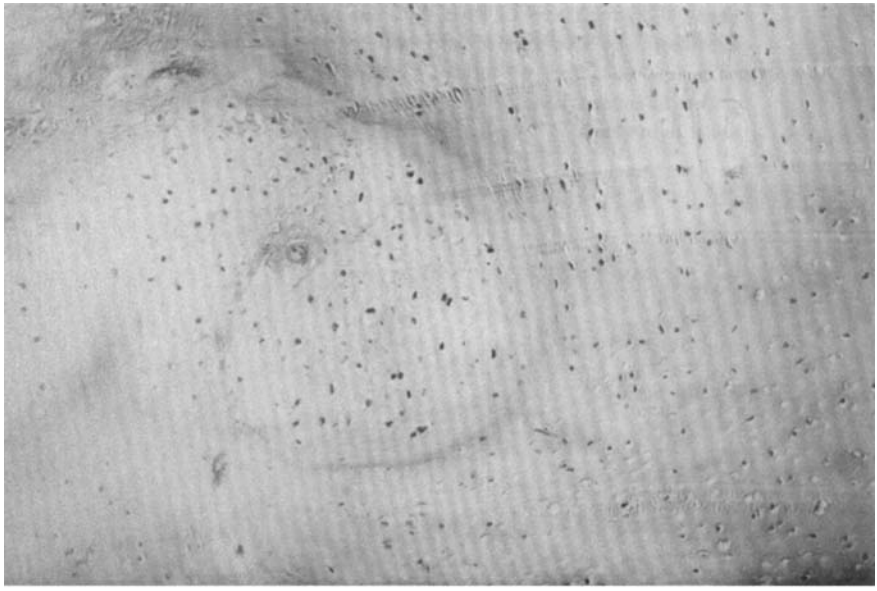
B. From zone c. Observation period: 12 weeks.
(Haemalun-azophloxine-saffron, x 150)

The picture shows an island of probably new cartilage formed within an area of necrotic cartilage.

C. From zone c (From the same transplant as the section shown in Figure 24 B).
Haemalun-azophloxine-saffron, x 150)

The picture shows several pale islands of probably new cartilage within darker regions of necrotic cartilage.

B



C

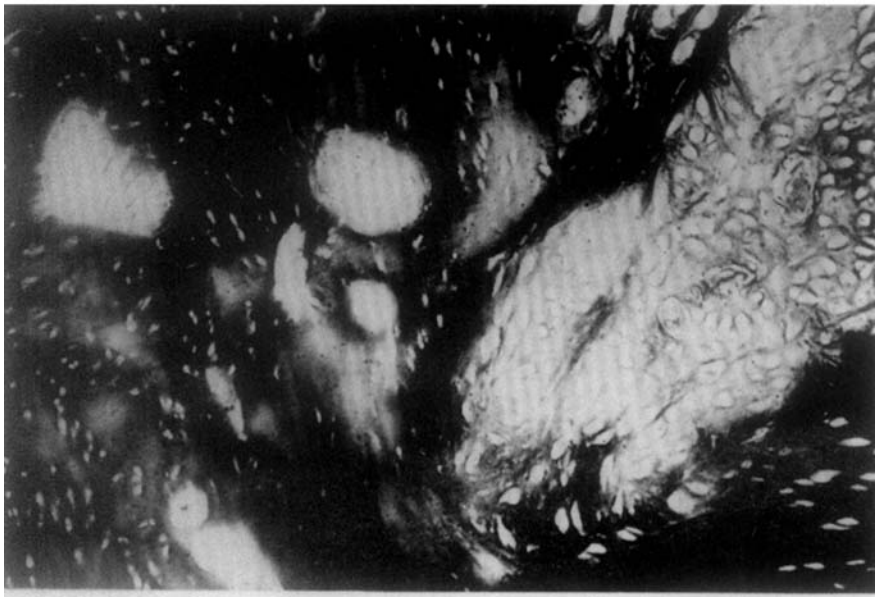
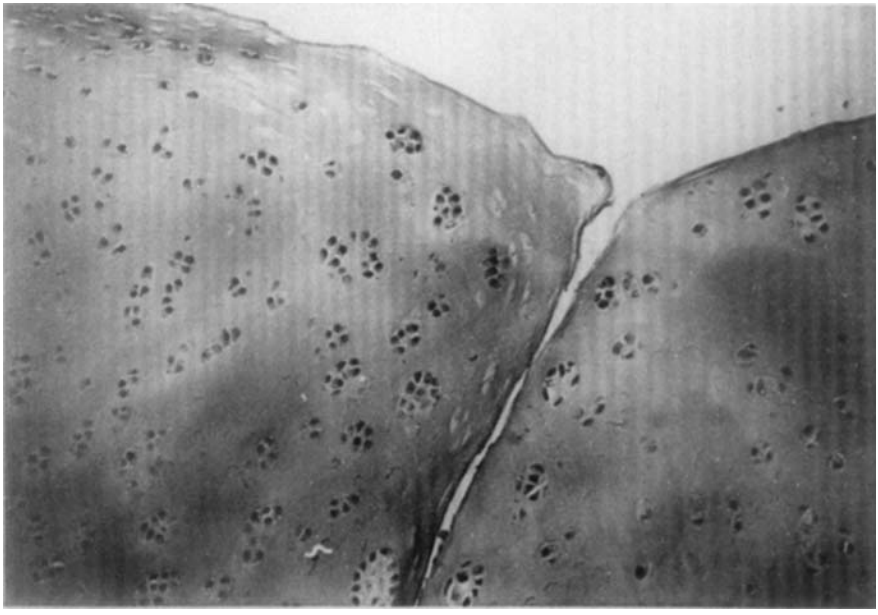


Figure 25. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Union joint cartilage/transplanted cartilage.

A. Observation period: 6 weeks. (detail from section shown in Figure 17 A) (Haemalun-azophloxine-saffron, x 150)

A very narrow cleft is seen between the transplanted cartilage (to the right) and the joint cartilage but there are no signs of union. Cluster cells are seen in the marginal area of both the transplant and the joint cartilage.



B. Observation period: 2 weeks.

(Haemalun-azophloxine-saffron, x 150)

The picture shows a specimen where there is a cleft between the transplant (to the right) and the the joint cartilage. The cleft, the width of which varies between 0.2 and 0.6 mm is filled with a relatively undifferentiated connective tissue. In the marginal region of the joint cartilage necrotic cells are seen and peripherally to these cluster cells are found. In the marginal zone of the transplant there are no cells, centrally to this area double cells and some cells containing four nuclei within the same capsula are seen.

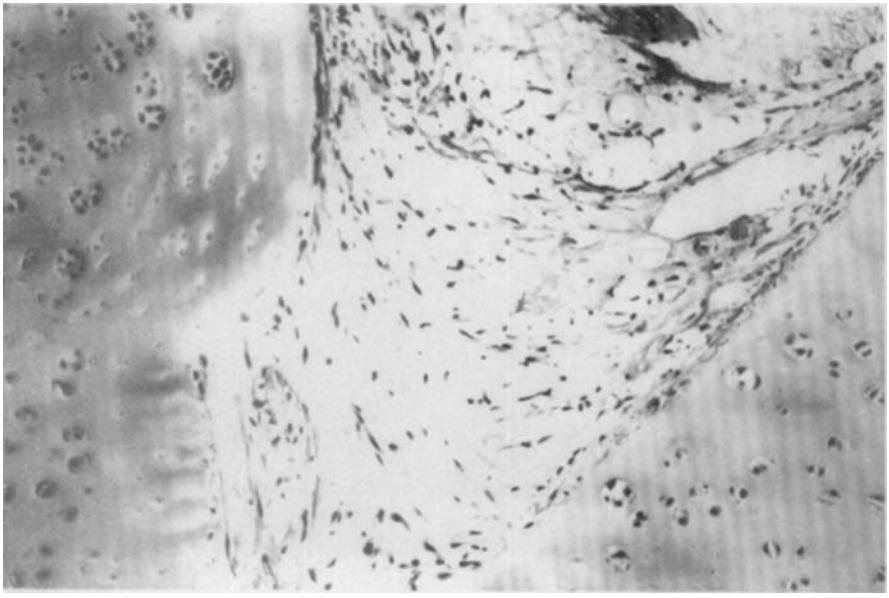


Figure 25. (Continued)

C. Observation period: 12 weeks (detail from section shown in Figure 18 A).

(Haemalun-azophloxine-saffron, x 40)

The picture shows joint cartilage (to the left) connected to transplanted cartilage by fibrorcartilage. The width of the fibrocartilaginous zone is about 0.5 mm.

Figure 26. Histological sections from osteochondral transplants to the joint surface of the femoral condyles (Tr.pl. A).
Union transplanted cartilage/bone bed.

A. Observation period: 2 weeks.
(Haemalun-azophloxine-saffron, x 150)

The picture shows a relatively undifferentiated connective tissue between the transplanted cartilage and the bone bed. In the peripheral region of the transplanted cartilage a narrow necrotic zone is seen and centrally to this there are many cells that contain two or more nuclei within the same capsule.

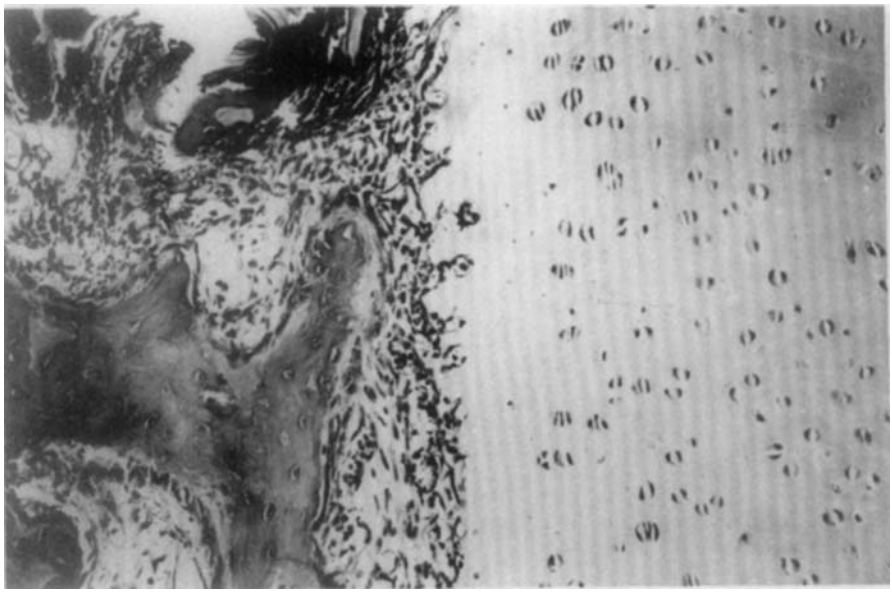


Figure 26. (Continued)

B. Observation period: 6 weeks.
(Haemalun-azophloxine-saffron, x 150)

The space between the transplanted cartilage and the bone tissue is now filled with fibrocartilage.

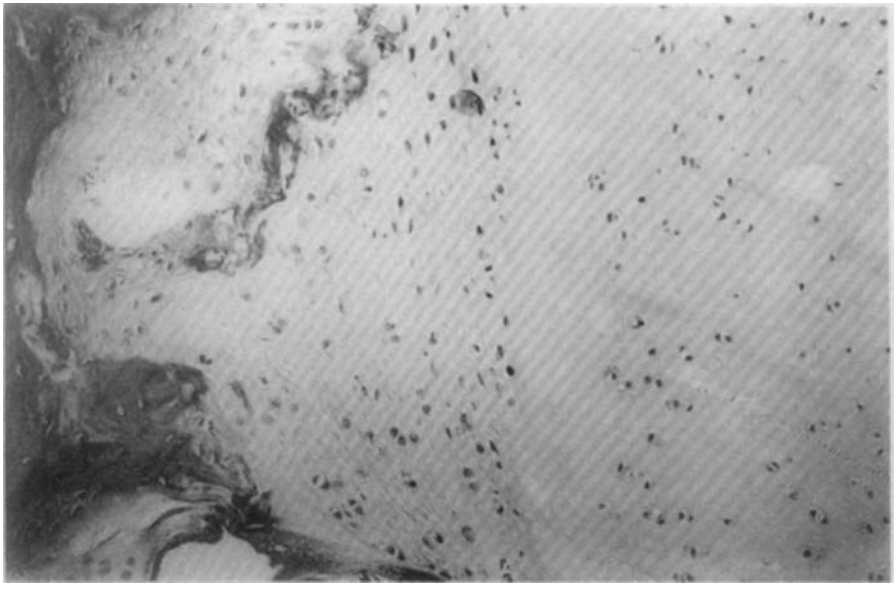


Figure 27. Histological sections from osteochondral transplants outside the joint surface (Tr.pl. B).

A. From the basal part of a transplant of 2 weeks' observation.
(Haemalun-azophloxine-saffron, x 40)

A small cartilage area is seen below a large cartilage area. Examination at greater magnification revealed that the former consisted of remnants of a necrotized growth plate whereas the latter contained a vital growth plate.

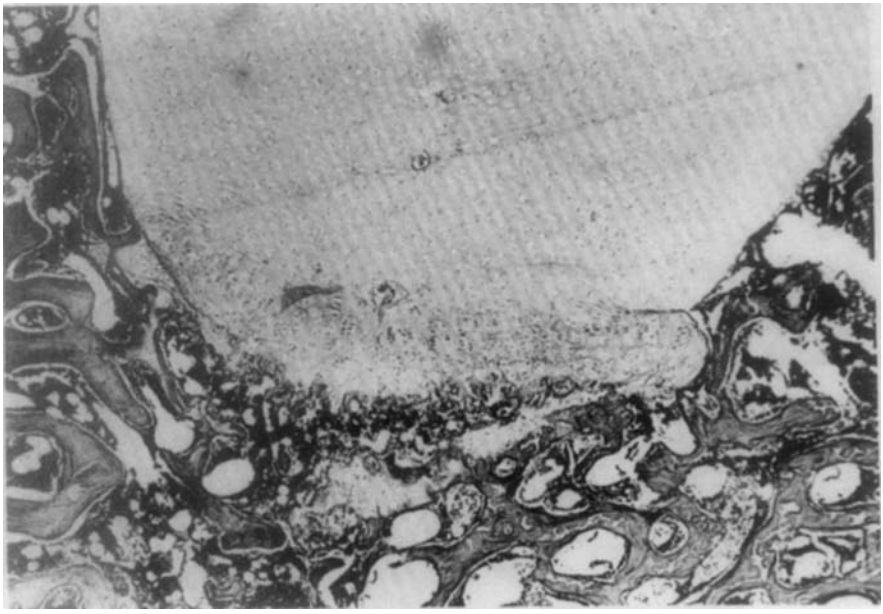
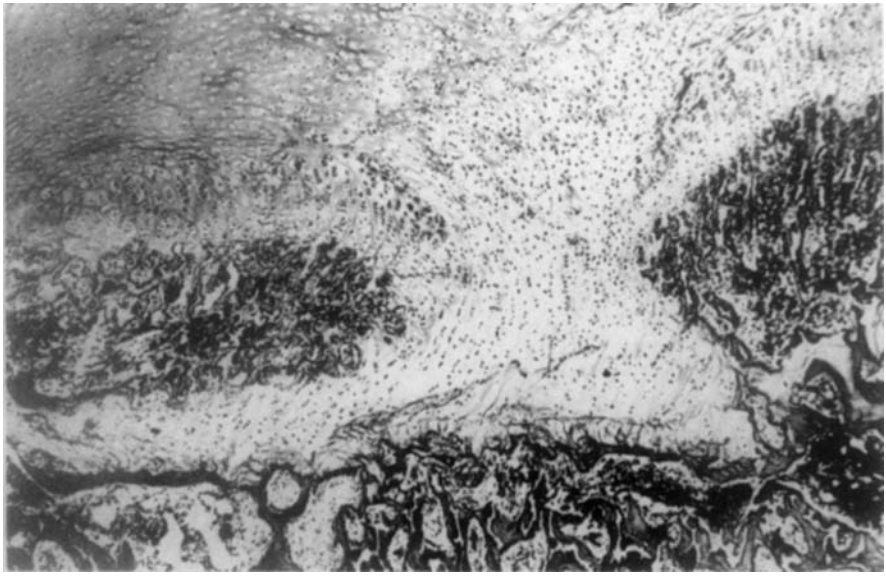


Figure 27.(Continued)

B. From the basal part of a transplant of 2 weeks' observation.
(Haemalun-azophloxine-saffron, x 40)

The cartilage at the bottom of the picture represents a necrotized growth plate. The highly cellular zone in the basal area of the cartilage above represents a vital growth plate. The ossification has been resumed in the vital growth plate. Note: The cell columns of the necrotic growth plate are converging upwards.



C. From the basal part of a transplant of 12 weeks' observation.

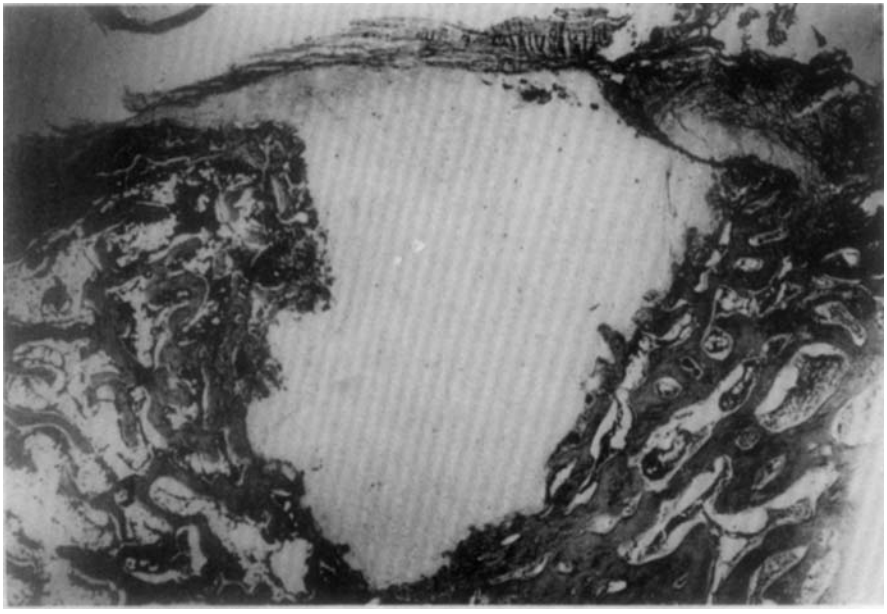
(Haemalun-azophloxine-saffron, x 150)

There are still columnar arrangement of the cells, swelling of the cells at the bottom of the columns, and enchondral ossification.

Figure 28. Histological sections from osteochondral transplants outside the joint surface (Tr.pl. B). Observation period: 12 weeks.

A. General view of a transplant.
(Haemalun-azophloxine-saffron, x 15)

The picture shows a transplant of which the major part of the cartilage has not become ossified. The transplant has been implanted through the epiphyseal plate (to the right). On the left side the transplant is undergoing ossification (compare Figure 28 C). Note: A weakly azophloxine-stained area is seen within the left half of the transplant (compare Figure 28 B and C); this represents a necrotic region.



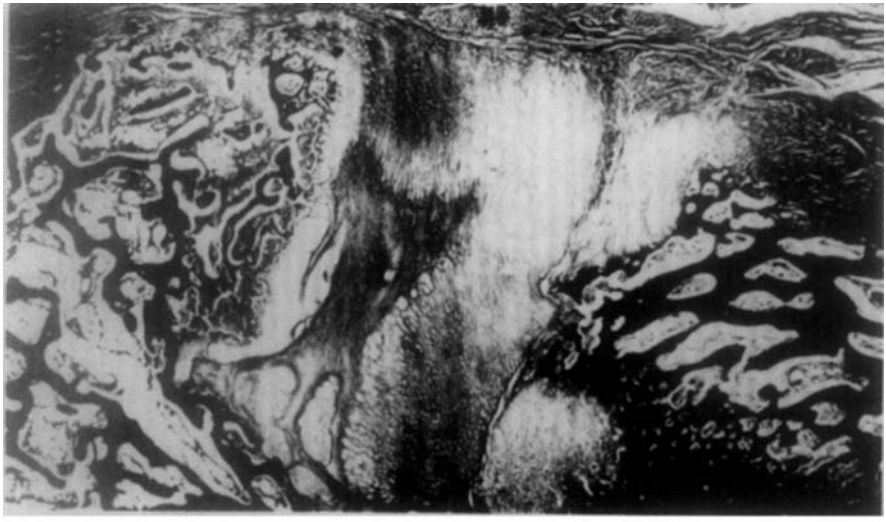
B. From approximately the same region as the section shown in Figure 28 A. (Gomori, x 15)

The Gomori staining demonstrates a clear demarcation of the central necrotic area (compare Figure 28 A and C). Further the epiphyseal plate is distinctly demarcated (to the right).

C. Detail from the left part of the section shown in Figure 28 A.
(Haemalun-azophloxine-saffron, x 150)

Proliferative cartilage cells and swollen cells are seen adjacent to a necrotic region to the right. To the left enchondral ossification takes place.

B



C

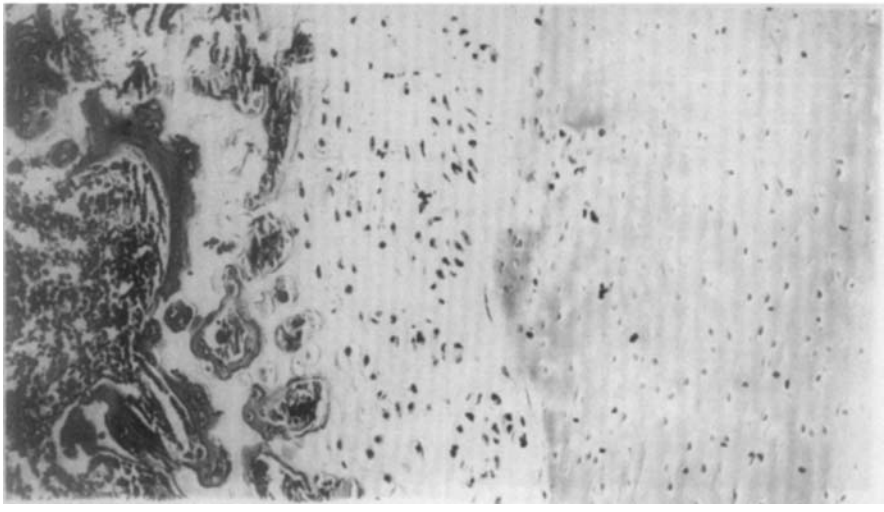


Figure 29. Histological sections from osteochondral transplants outside the joint surface (Tr.pl. B).
Observation period: 12 weeks.

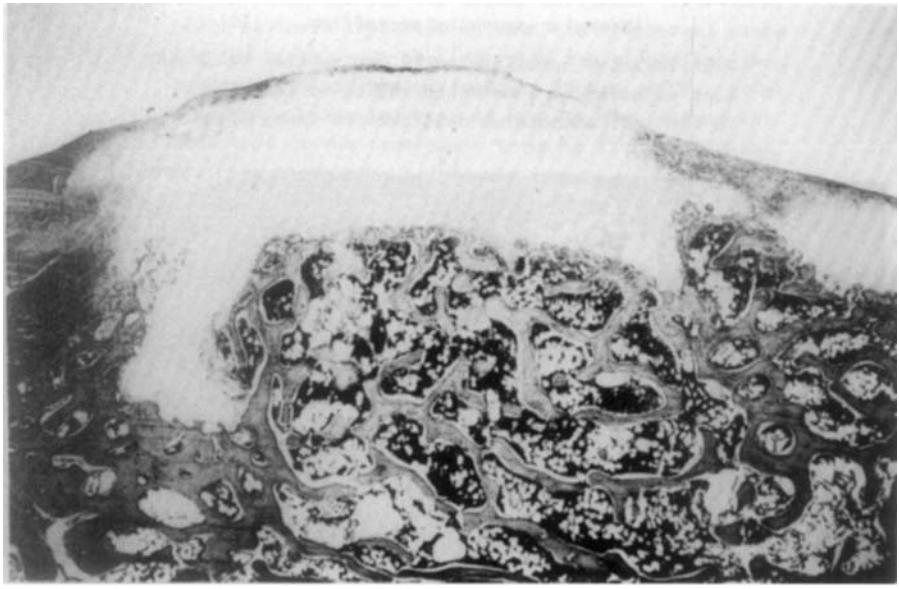
A. General view of a transplant. (Haemalun-azophloxine-saffron, x 15)

The picture shows a transplant in which the major part of zone c has become ossified.

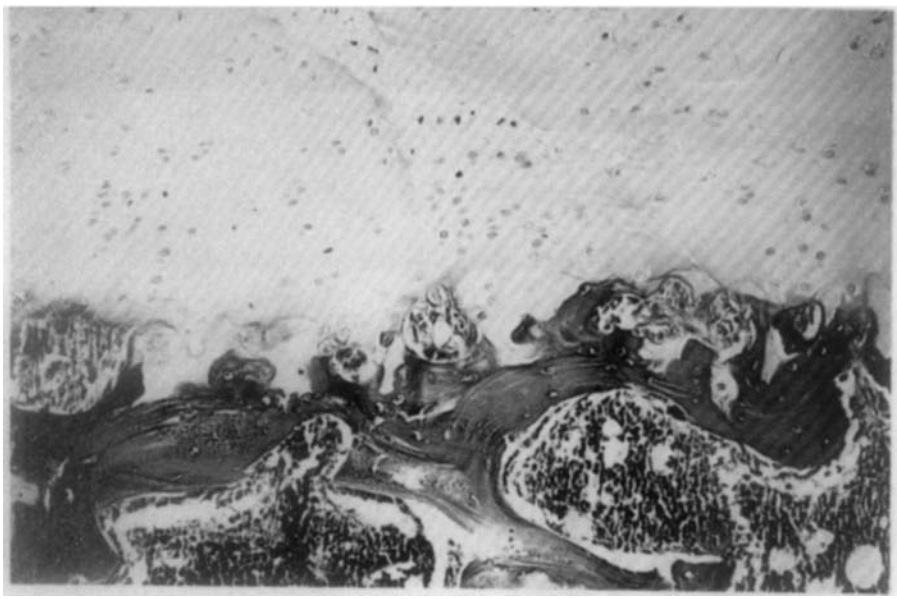
B. Detail from the basal central part of the section shown in Figure 29 A. (Haemalun-azophloxine-saffron, x 150)

Vital cartilage cells and ossification without any preceding swelling of the cartilage cells in the zone of ossification are seen.

A



B



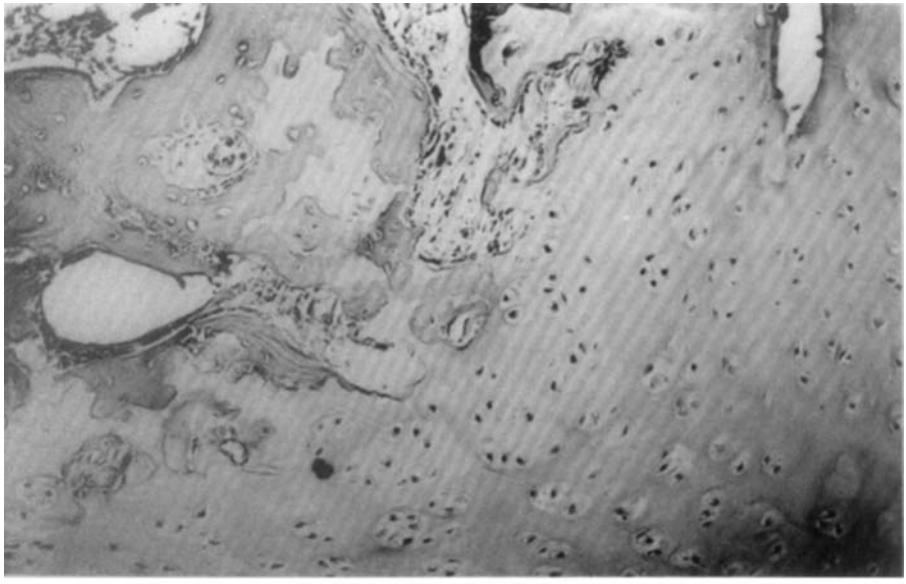
C. From the peripheral part of a transplant.
(Haemalun-azophloxine-saffron, x 150)

The picture shows ossification in an area which contains double cells and cluster cells. The cartilage cells in the zone of ossification are markedly swollen.

D. From the same region as the section shown in Figure 29 C.
(Alcian green, x 150)

The swollen cells contain strongly Alcian-stained cytoplasm.

C



D

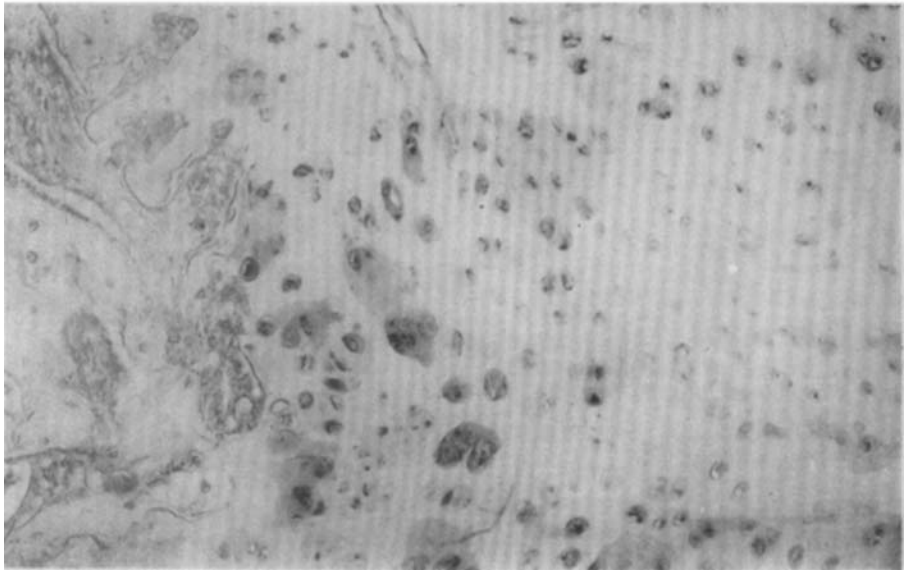
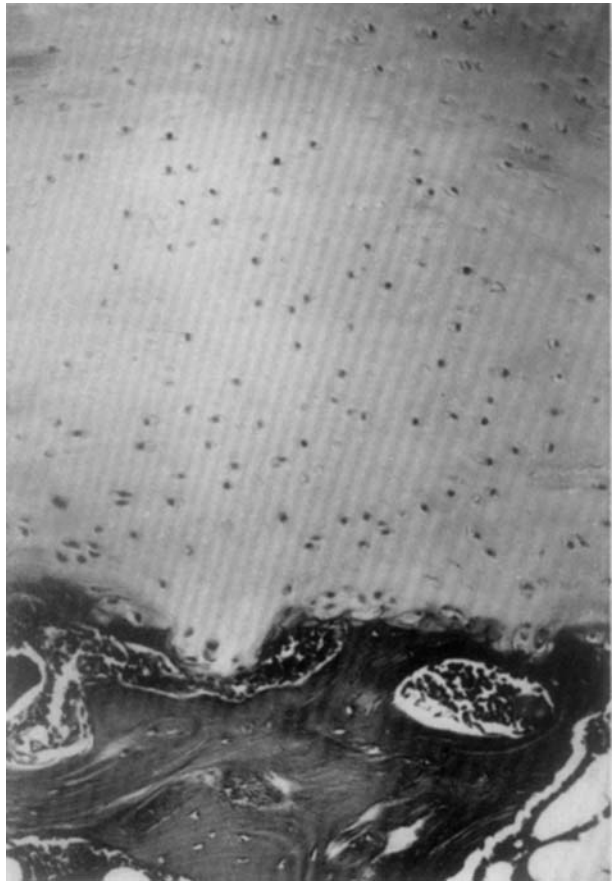


Figure 30. Histological sections from osteochondral transplants outside the joint surface (Tr.pl. B). Observation period: 6 months.

A. From the basal part of the persisting cartilage of a transplant.
(Haemalun-azophloxine-saffron, x 150)

There is no swelling of the cartilage cells.



B. General view of a transplant.
(Haemalun-azophloxine-saffron, x 15)

The picture shows a completely ossified transplant beneath a thick layer of connective tissue.

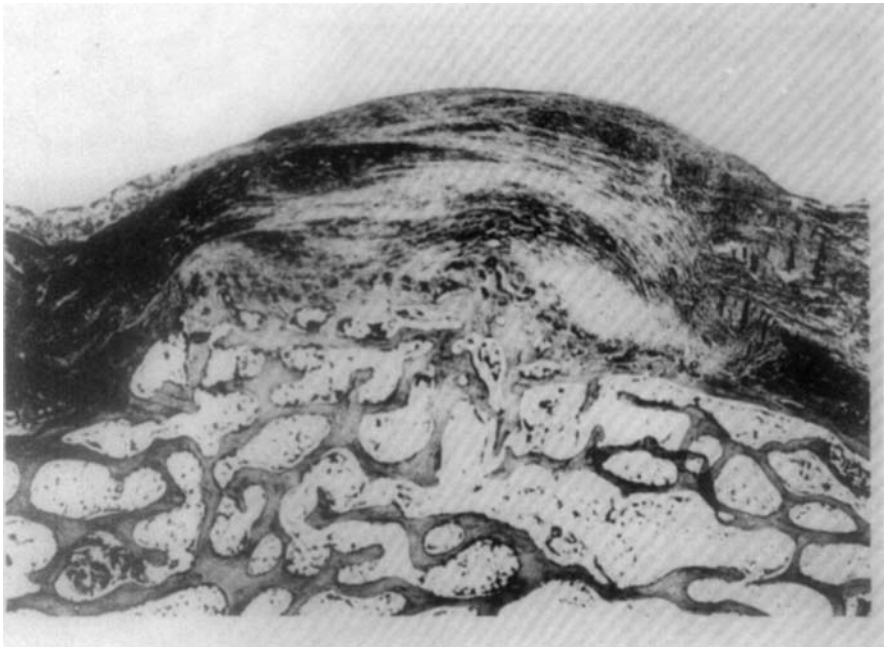


Figure 31. Histological sections from osteochondral transplants outside the joint surface (Tr.pl. B).

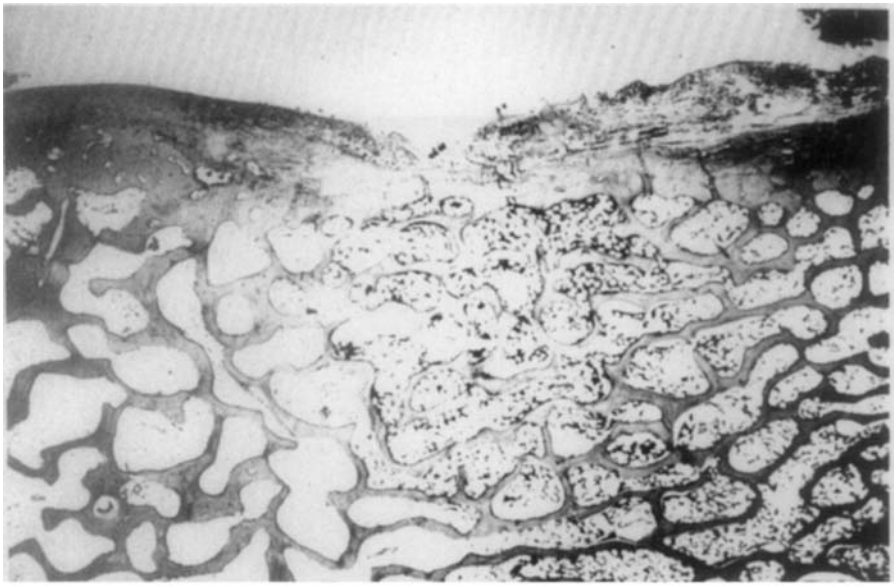
A. Observation period: 14 months.
(Haemalun-azophloxine-saffron, x 15)

The picture shows a transplant which has become completely ossified.

B. Observation period: 14 months.
(Haemalun-azophloxine-saffron, x 15)

Only a small part of the superficial zone of this transplant is still unossified. The transplant has obviously been markedly protruding.

A



B

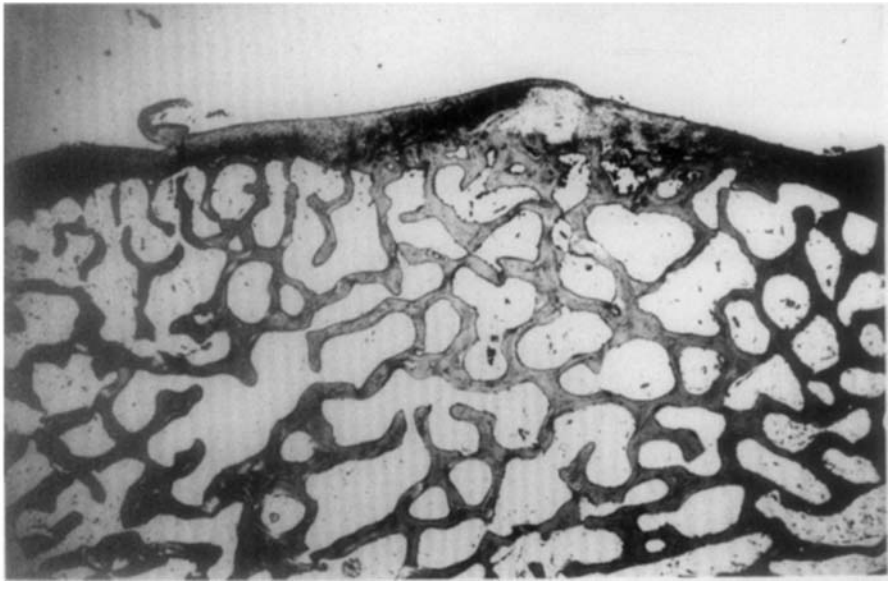


Figure 32. Histological sections from osteochondral transplants outside the joint surface (Tr.pl. B). Presence of vessels and connective tissue within vital cartilage regions.

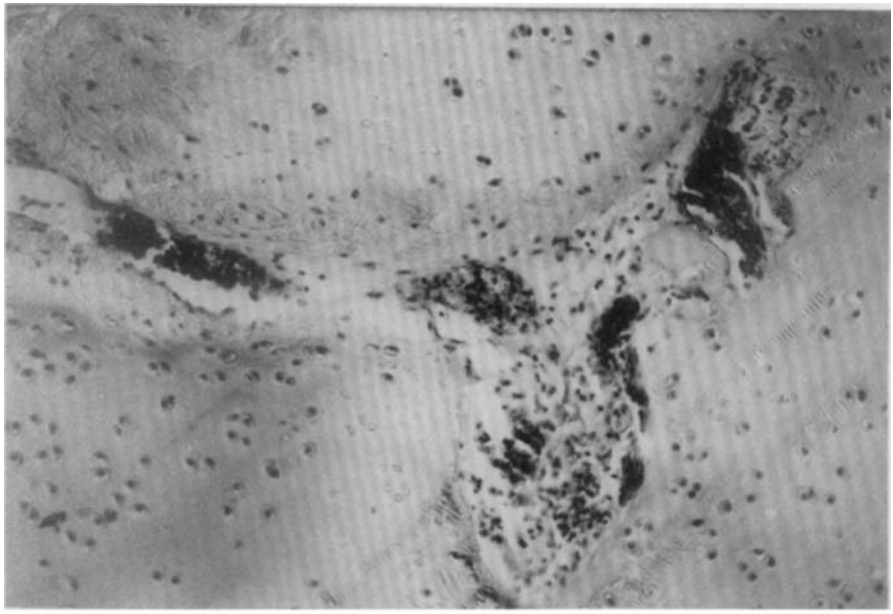
A. From the transition between zones a and b.
Observation period: 2 weeks.
(Haemalun-azophloxine-saffron, x 150)

The perivascular connective tissue contains elongated, fusiformed cells.

B. From zone a.
Observation period: 6 weeks.
(Haemalun-azophloxine-saffron, x 150)

There are no signs of ossification around the vessel.

A



B

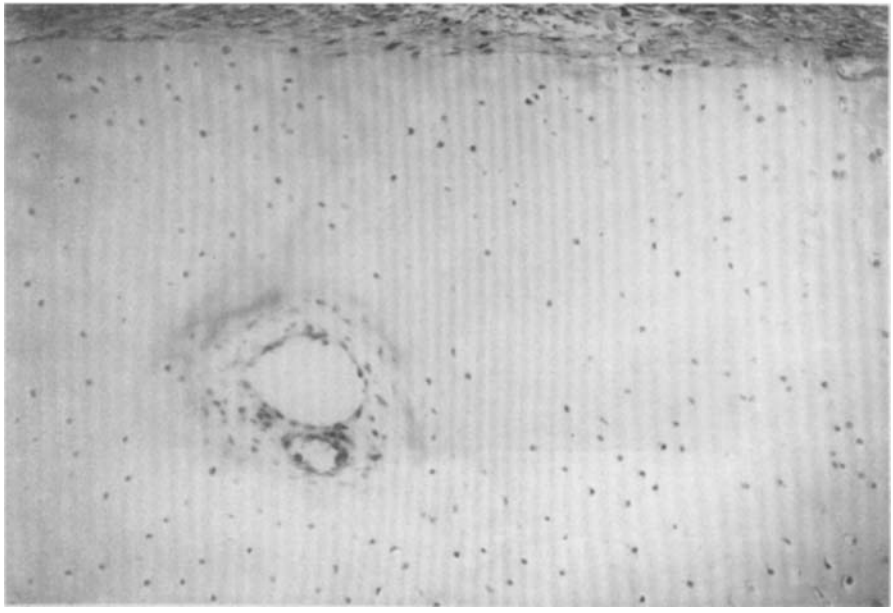
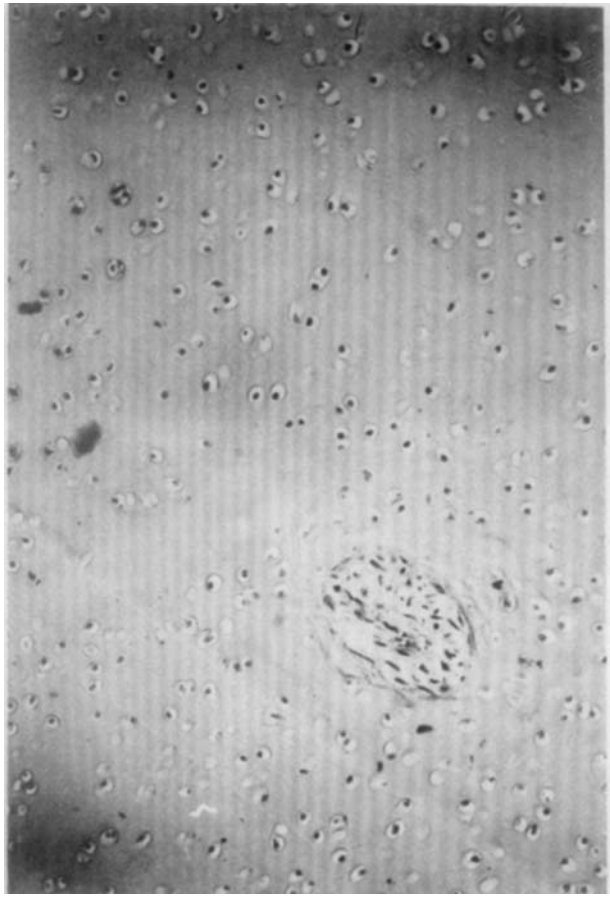


Figure 33. Histological sections from osteochondral transplants outside the joint surface (Tr.pl. B). Changes within necrotic cartilage.

A. From zone c. Observation period: 2 weeks. (Haemalun-azophloxine-saffron, x 150)

An islet of connective tisse is seen within a necrotic cartilage region.



B. From zone c. Observation period: 6 weeks.
(Haemalun-azophloxine-saffron, x 150)

The picture shows an islet of vital cartilage within a necrotic cartilage region.

Figure 33. (Continued)

C. From the growth plate/basal part of zone c.
Observation period: 6 weeks.
(Haemalun-azophloxine-saffron, x 150)

The picture shows vital, slightly fibrous cartilage in a region corresponding to a necrotized growth plate and within necrotic regions of zone c. Down to the left the cells of this cartilage are slightly swollen and ossification takes place.

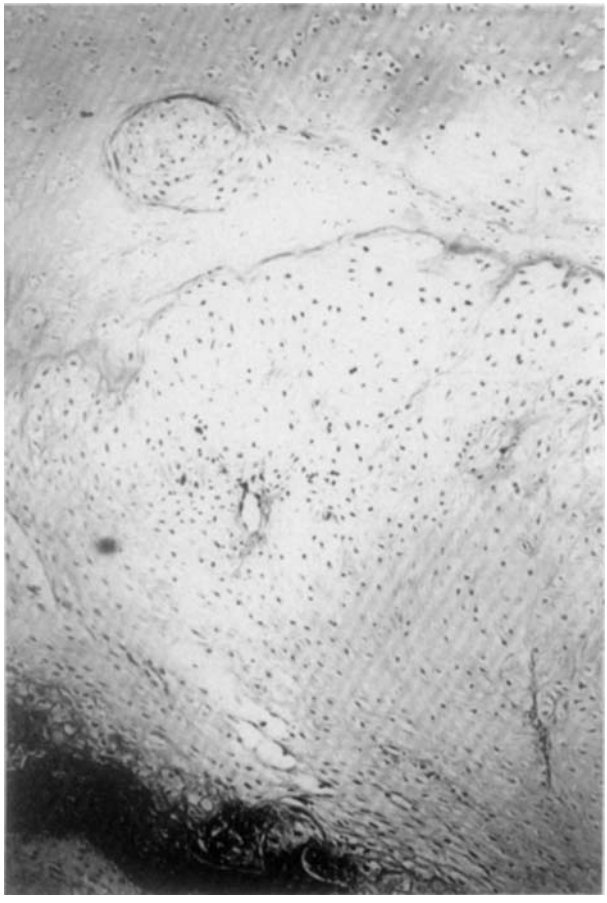


Figure 34. Histological sections from control defects in the joint surface of the femoral condyles partly filled with osseous transplants (Tr.pl. C). Observation period: 2 weeks.

A. General view of a defect. (Haemalun-azophloxine-saffron, x 15)

The defect above the transplanted bone fragment has been partly filled with granulation tissue. The most basal part of this granulation tissue is highly vascular.

B. Detail from section shown in Figure 34 A. (Haemalun-azophloxine-saffron, x 150)

The picture shows that the connective tissue in the superficial part of the defect contains relatively undifferentiated connective tissue cells with oval or fusiformed nuclei. In the joint cartilage double cells are seen peripheral to a necrotic marginal zone.

A

B

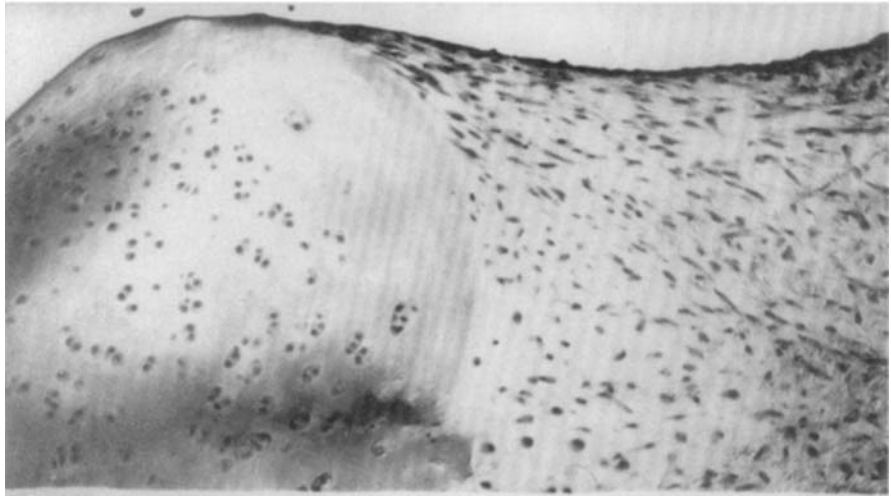


Figure 34. (Continued)

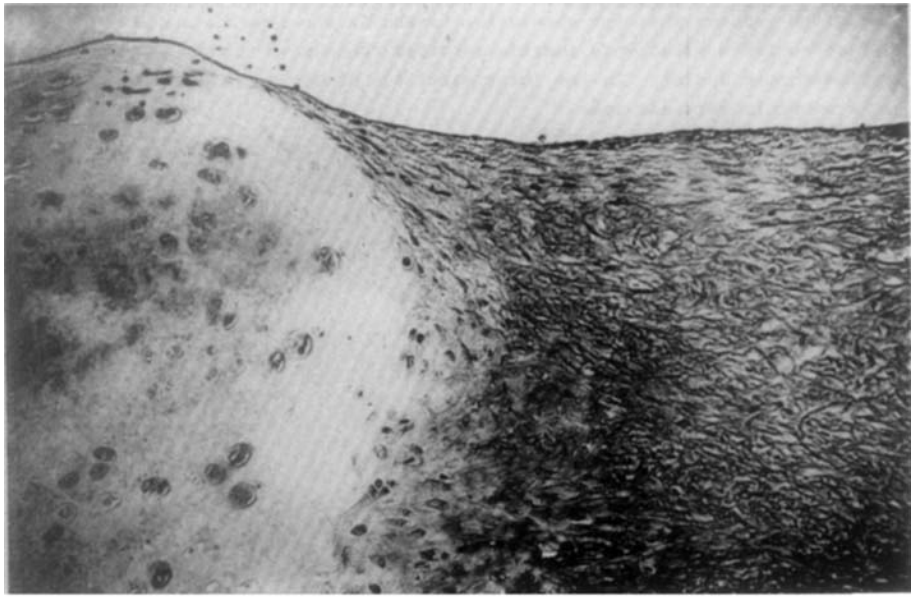
C. From the same region as shown in Figure 34 A. (Alcian green, x 150)

The granulation tissue is clearly Alcian-positive.

D. From the transplanted bone tissue. (Haemalun-azophloxine-saffron, x 150)

The central parts of the bone trabeculae contain necrotic osteocytes (empty bone lacunae). The superficial parts of the trabeculae contain vital osteocytes (well-stained nuclei) and lined with osteoblasts.

C



D

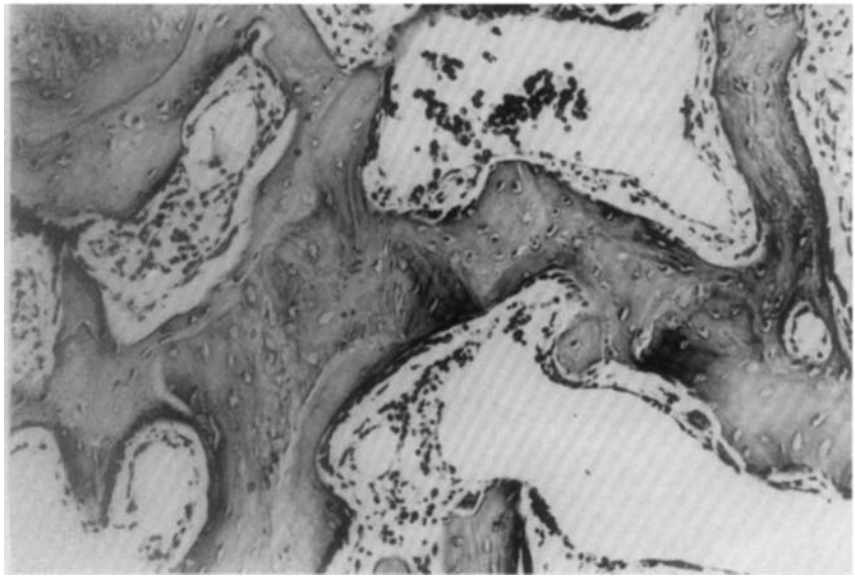


Figure 35. Histological sections from control defects in the joint surface of the femoral condyles partly filled with osseous transplants (Tr.pl. C). Observation period: 6 weeks.

A. From the transitional area between joint cartilage/granulation tissue.
(Haemalun-azophloxine-saffron, x 150)

The cells of the granulation tissue are more round than at the 2 weeks' observation. Peripheral to a narrow necrotic zone some cluster cells are seen within the joint cartilage.

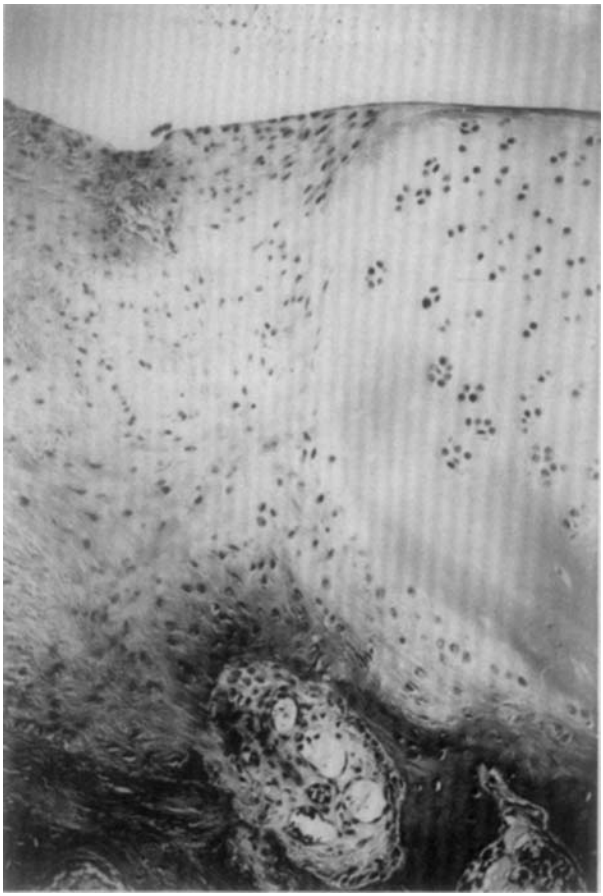


Figure 35. (Continued)

B. From the basal part of the granulation tissue of a defect.
(Haemalun-axophloxine-saffron, x 150)

The picture shows a gradual transition from connective tissue cells (up to the left) to swollen cartilage cells. The cartilage undergoes ossification.

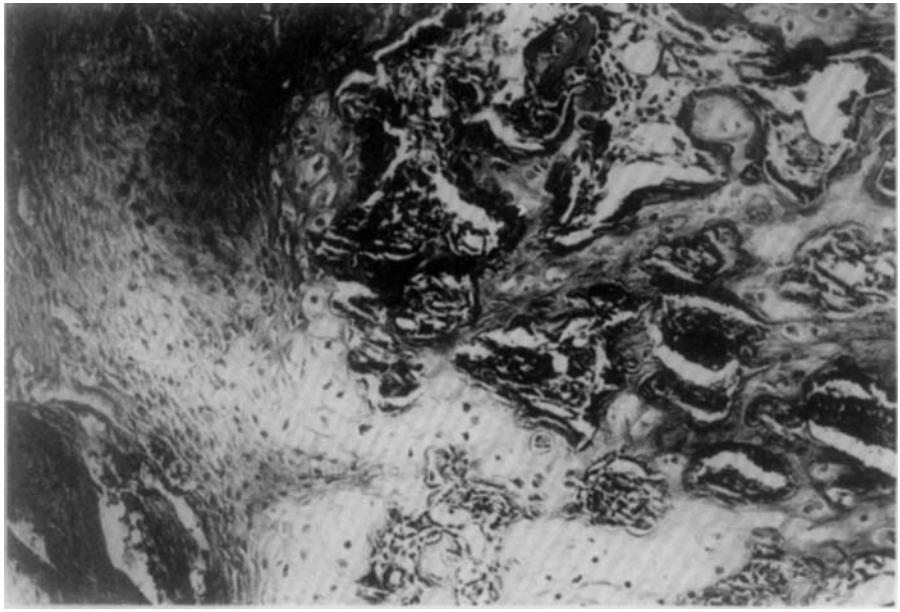


Figure 36. Histological sections from control defects in the joint surface of the femoral condyles partly filled with osseous transplants (Tr.pl. C). Observation period: 6 weeks.

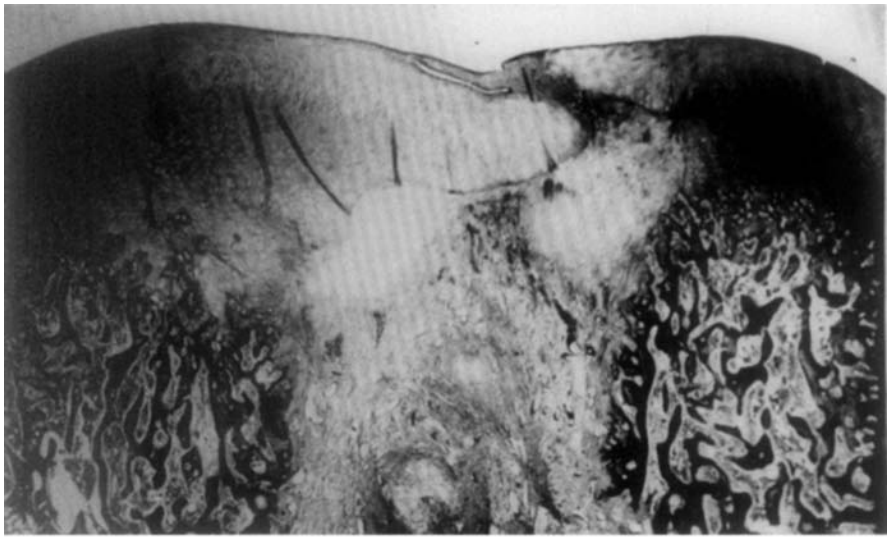
A. General view of a defect (the same defect as shown macroscopically in Figure 11 B) (Gomori, x 15)

The picture shows a defect where the joint cartilage has almost completely grown over the defect. Note the distinct demarcation from the underlying tissue.

B. Detail from the same defect as shown in Figure 36 A. (Haemalun-azophloxine-saffron, x 150)

Up to the left some part of the cartilage which has grown over the defect is seen. This cartilage contains cluster cells above a narrow zone of necrotic cells. Down to the right of this cartilage fibrocartilage is seen.

A



B

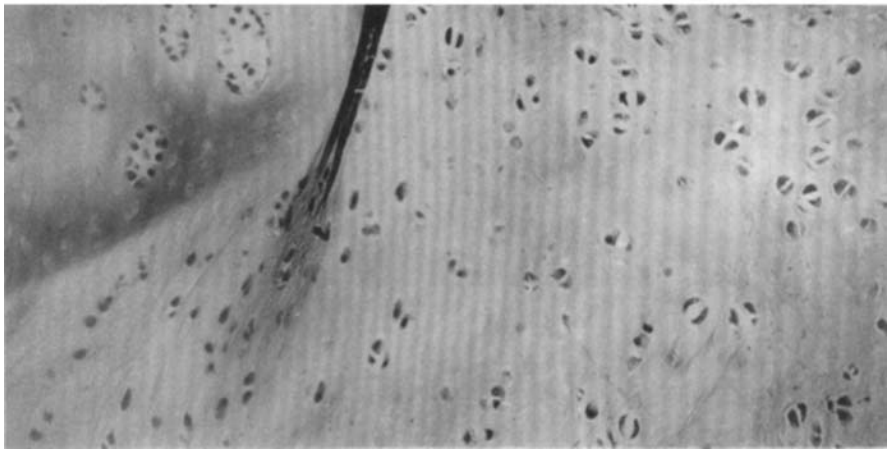
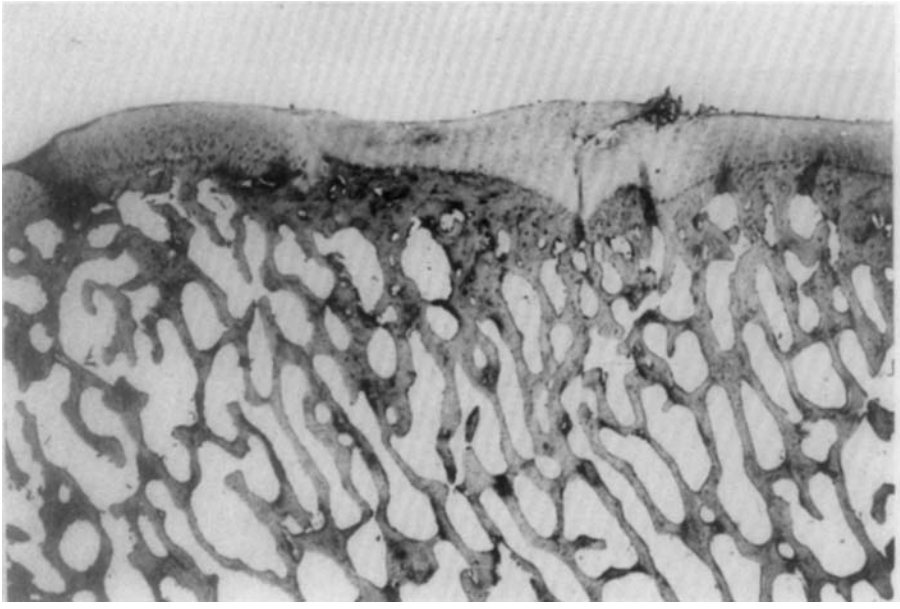


Figure 37. Histological sections from control defects in the joint surface of the femoral condyles partly filled with osseous transplants (Tr.pl. C). Observation period: 14 months.

A. General view of a defect.
(Haemalun-azophloxine-saffron, x 15)

The height of the fibrocartilage filling the central part of the original defect is in the greater part of the defect lower than that of the joint cartilage.



B. Detail from the fibrocartilage in the central part of the defect shown in Figure 37 A.
(Haemalun-azophloxine-saffron, x 150)

The picture shows that the matrix is still clearly fibrous. In the deeper part a basophilic zone is seen and this zone closely resembles the basal calcified zone of the articular cartilage (compare Figures 22 C and 23 C).

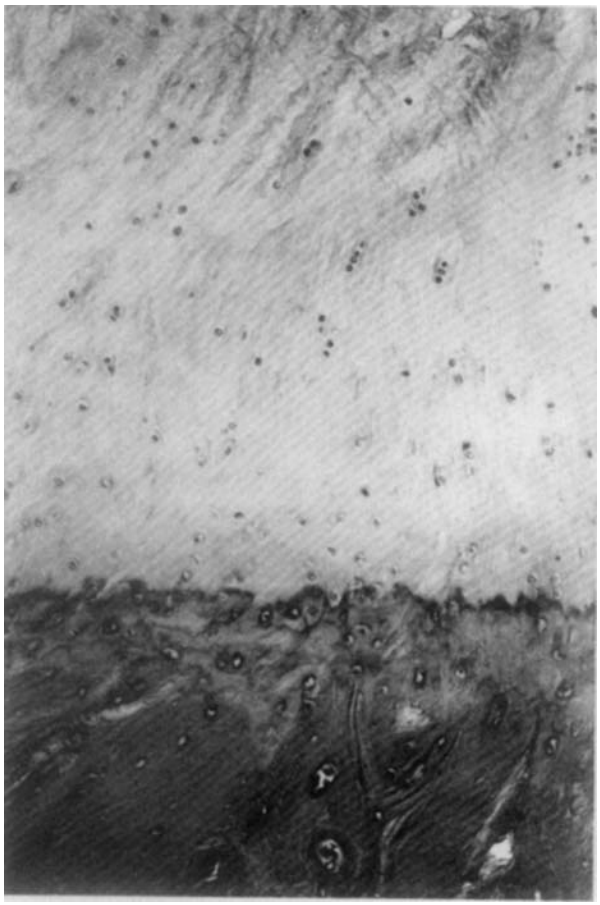
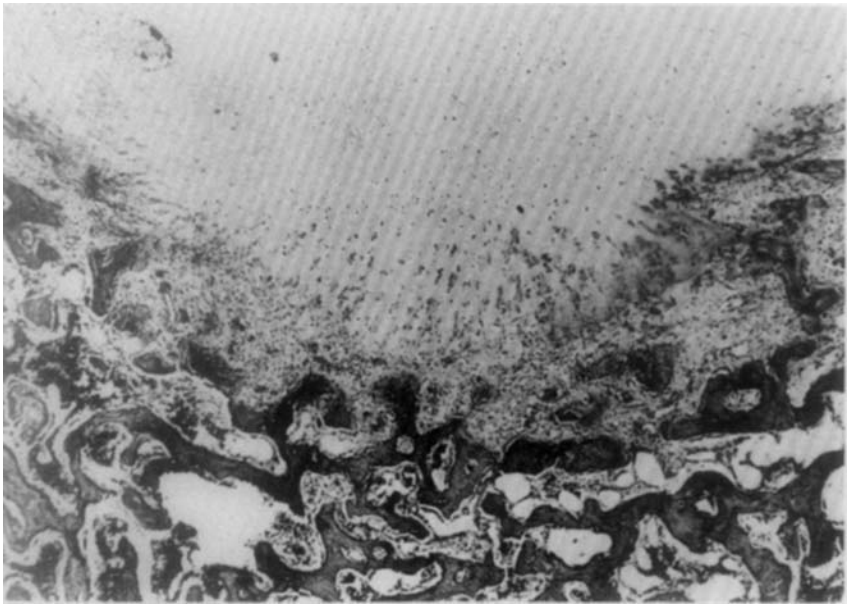


Figure 38. Histological sections from control defects in the joint surface of the femoral condyles filled with osseous transplants and devitalized cartilage (Tr.pl. D).
Observation period: 2 weeks.

A. From the basal part of the devitalized cartilage.
(Haemalun-azophloxine-saffron, x 40)

The picture shows ingrowth of vascular connective tissue in the devitalized cartilage.



B. Detail from section shown in Figure 38 A.
(Haemalun-azophloxine-saffron, x 150)

Oval and fusiformed connective tissue cells are seen in the matrix septa between the columns of necrotic cells and below these cells.

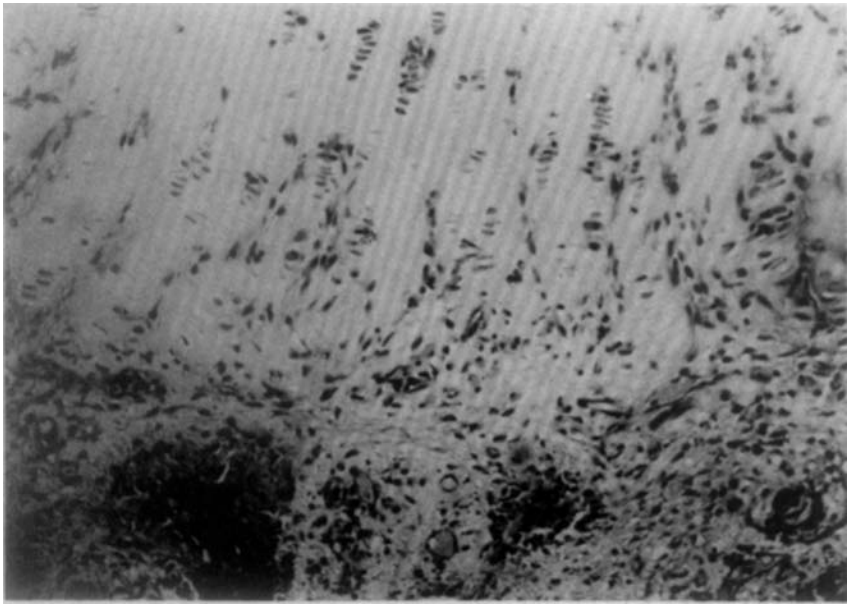
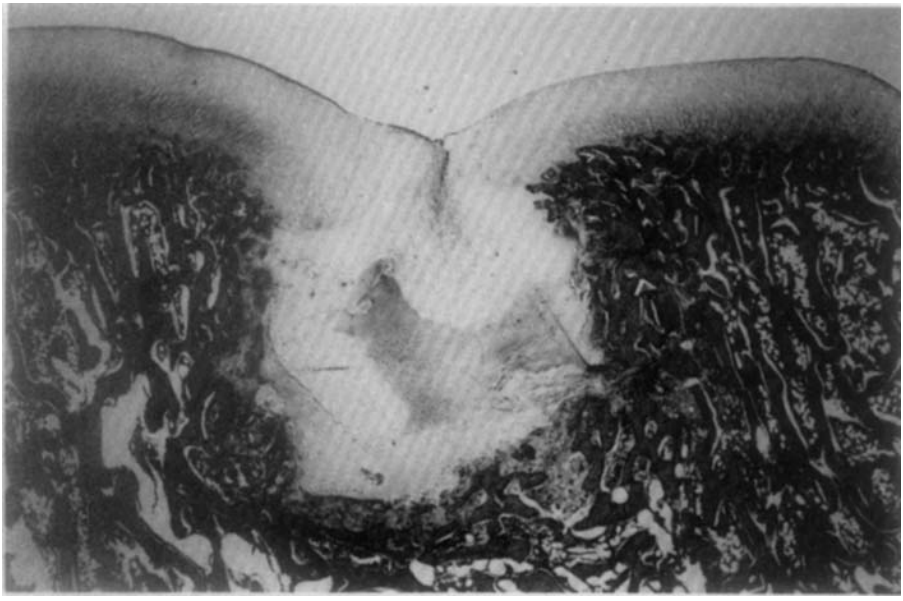


Figure 39. Histological sections from control defects in the joint surface of the femoral condyles filled with osseous transplants and devitalized cartilage (tr.pl. D).
Observation period: 6 weeks.

A. General view of a defect.
(Haemalun-azophloxine-saffron, x 15)

The picture shows a defect where great parts of the devitalized cartilage have been resorbed. The remnants of the devitalized cartilage which are seen centrally and basally are stained with azophloxine. Superficially the defect is covered with cartilage deriving from the adjacent joint cartilage (compare Figure 39 B). The surface corresponding to the original defect is depressed.



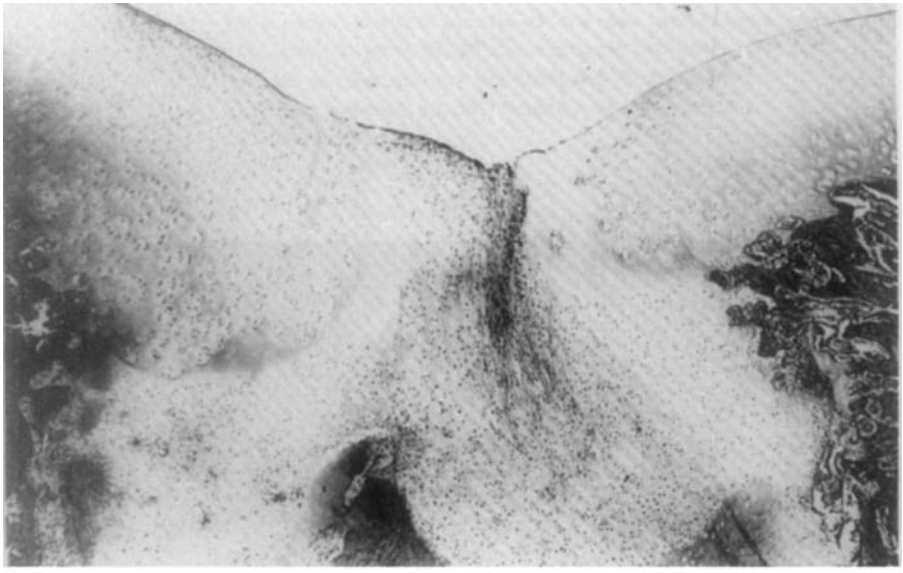
B. Detail from the superficial part of the defect shown in Figure 39 A.
(Haemalun-azophloxine-saffron, x 40)

The devitalized cartilage has been replaced by highly cellular cartilage centrally and beneath the ingrown joint cartilage. The latter contains plenty of cluster cells.

C. Detail from the devitalized cartilage of another defect.
(Haemalun-azophloxine-saffron, x 150)

The picture shows ingrowing connective tissue (in the middle) and formation of new cartilage (to the left). To the right devitalized cartilage is seen.

B



C

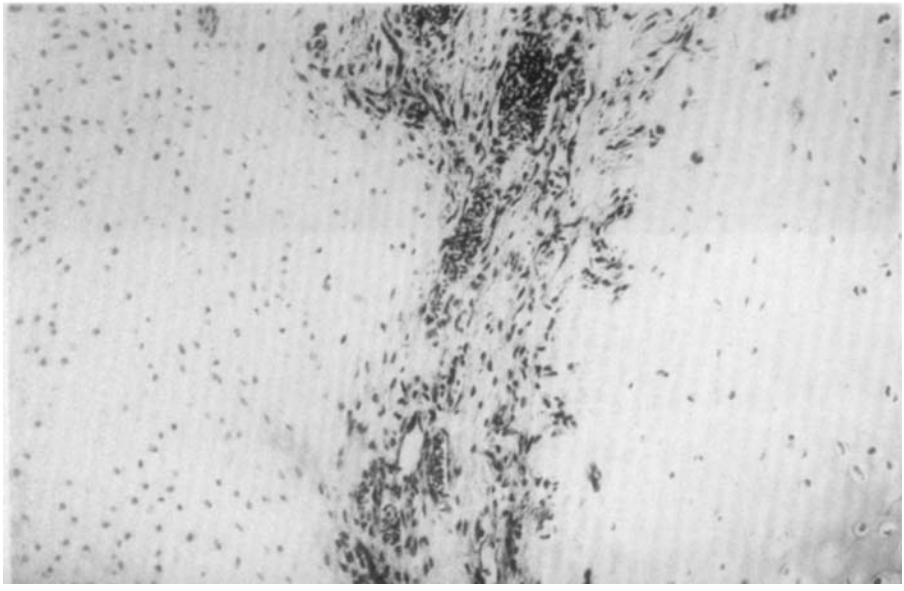


Figure 39. (Continued)

D. Detail from the peripheral deeper part of the defect shown in Figure 39 A and B.
(Haemalun-azophloxine-saffron, x 150)

The cells of the new cartilage are markedly swollen and ossification is seen within the cartilage.

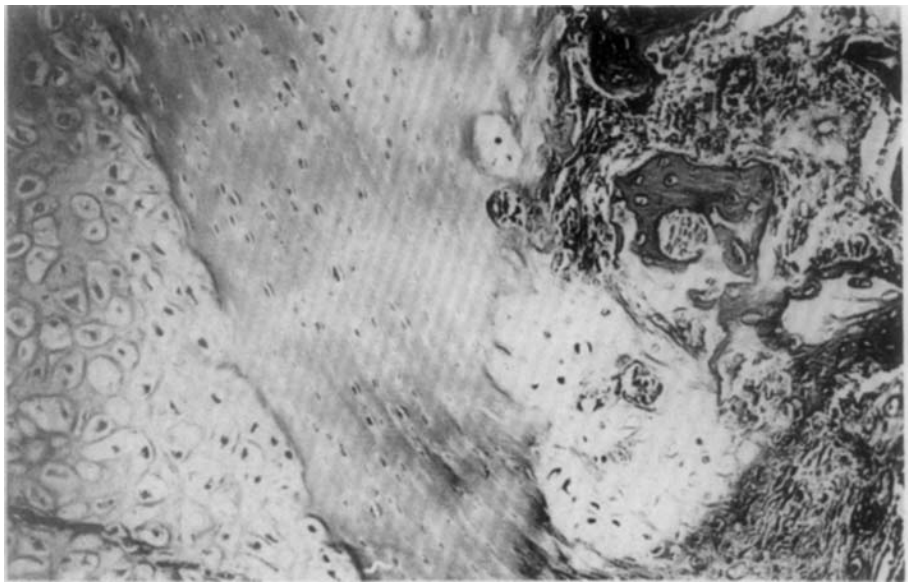


Figure 40. Histological sections from control defects in the joint surface of the femoral condyles filled with osseous transplants and devitalized cartilage (Tr.pl. D).

From the same defect as shown in Figure 39 A, B and D

(Alcian green, x 40)

The cells and the matrix of the new cartilage (compare Figure 39 B) are strongly Alcian-positive whereas the devitalized cartilage (at the bottom) is only weakly stained with Alcian green.

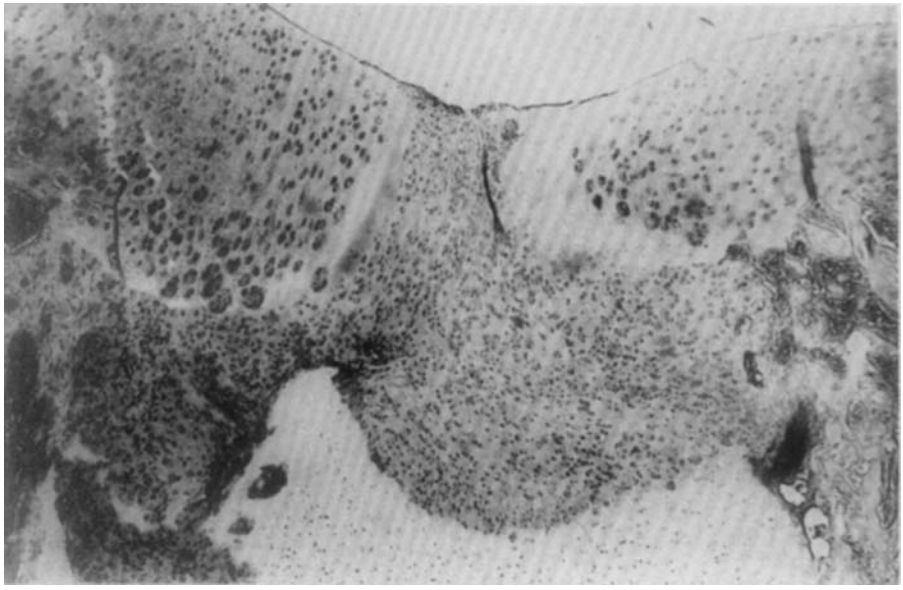
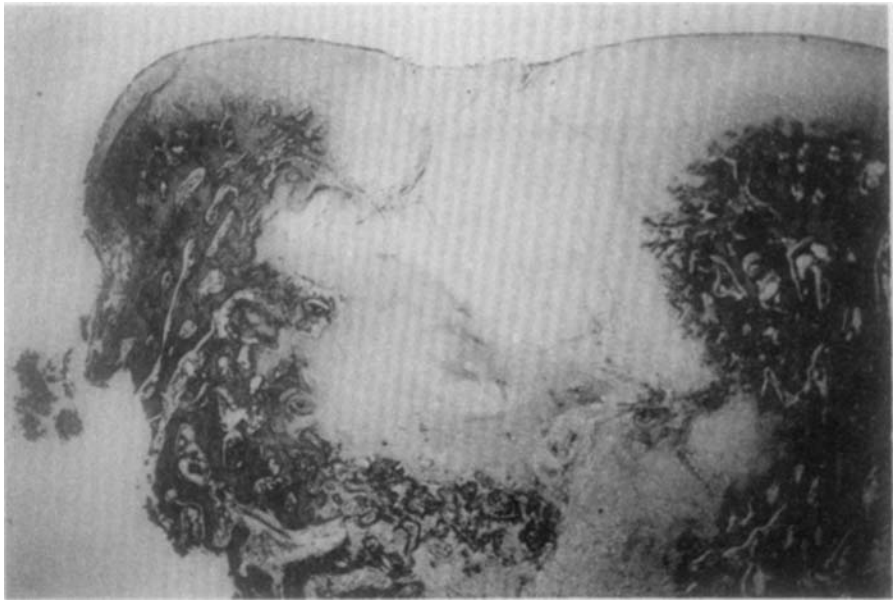


Figure 41. Histological sections from control defects in the joint surface of the femoral condyles filled with osseous transplants and devitalized cartilage (Tr.pl. D).
Observation period: 12 weeks.

A. General view of a defect.
(Haemalun-azophloxine-saffron, x 15)

The greater part of the devitalized cartilage in the defect has been resorbed; the remnants of it are still stained with azophloxine (down to the left). Below this cartilage ossification is seen within new cartilage (compare Figure 41 C).



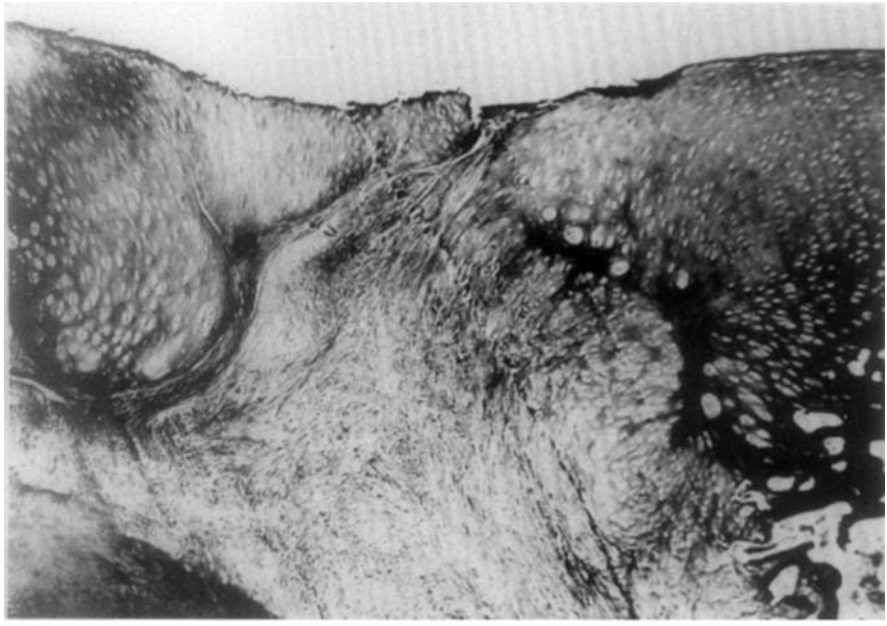
B. From the superficial part of the defect shown in Figure 41 A. (Gomori, x 40)

The picture shows a clear demarcation between ingrown joint cartilage and the cartilage in the central area. The latter appears slightly fibrous.

C. Detail from section shown in Figure 41 A. (Haemalun-azophloxine-saffron, x 150)

The picture shows in greater detail the enchondral ossification which takes place in the deeper part of the defect.

B



C

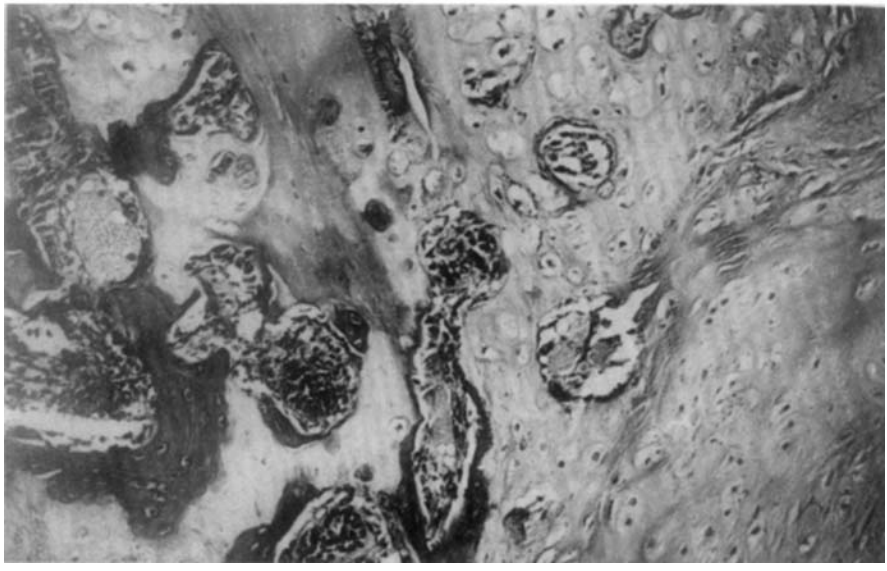


Figure 42. Histological sections from control defects in the joint surface of the femoral condyles filled with osseous transplants and devitalized cartilage (Tr.pl. D). Observation period: 6 months.

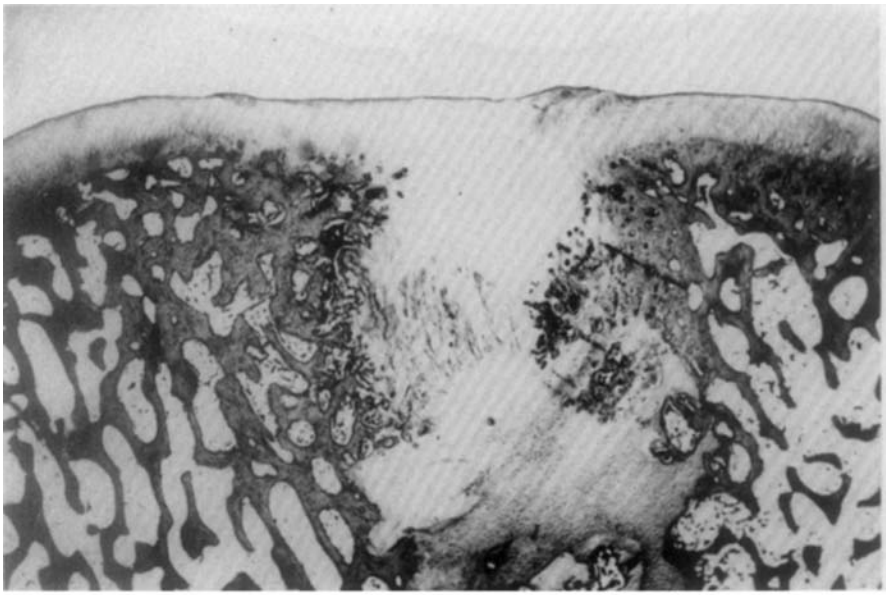
A. General view of a defect. (Haemalun-azophloxine-saffron, x 15)

The picture shows a defect where the greater part of the devitalized cartilage has been resorbed and where great parts of the defect have been filled with bone.

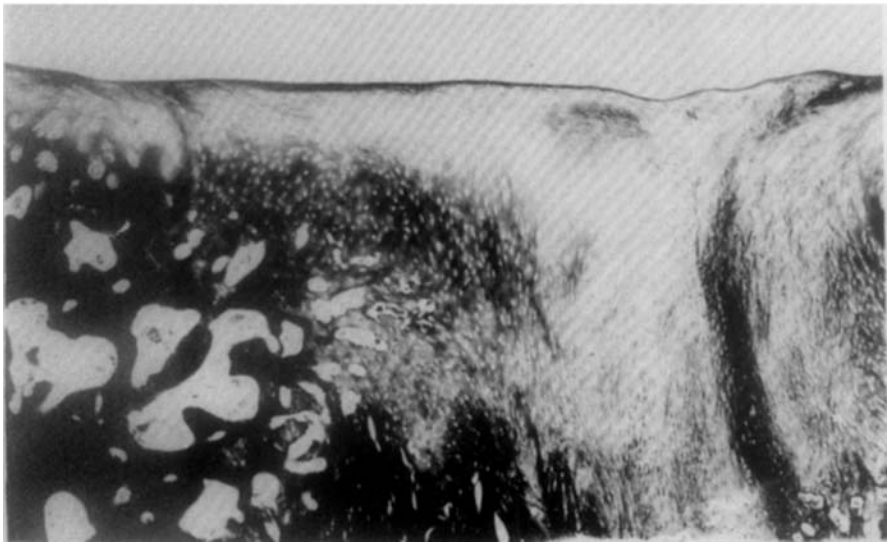
B. Detail from the superficial part of the defect shown in Figure 42 A. (Gomori, x 40)

This staining clearly demonstrates the border between the cartilage filling the central part of the defect and the joint cartilage (to the left).

A



B



C. Detail from the superficial part of the defect shown in Figure 42 A (from the area at the right side of the joint cartilage demonstrated in Figure 42 B).
(Haemalun-azophloxine-saffron, x 150)

The cartilage appears hyaline.

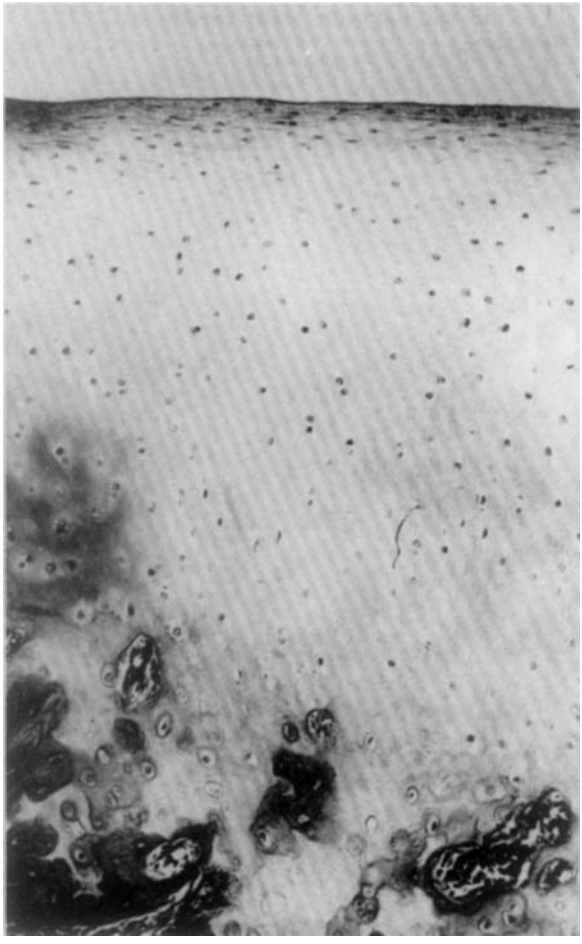
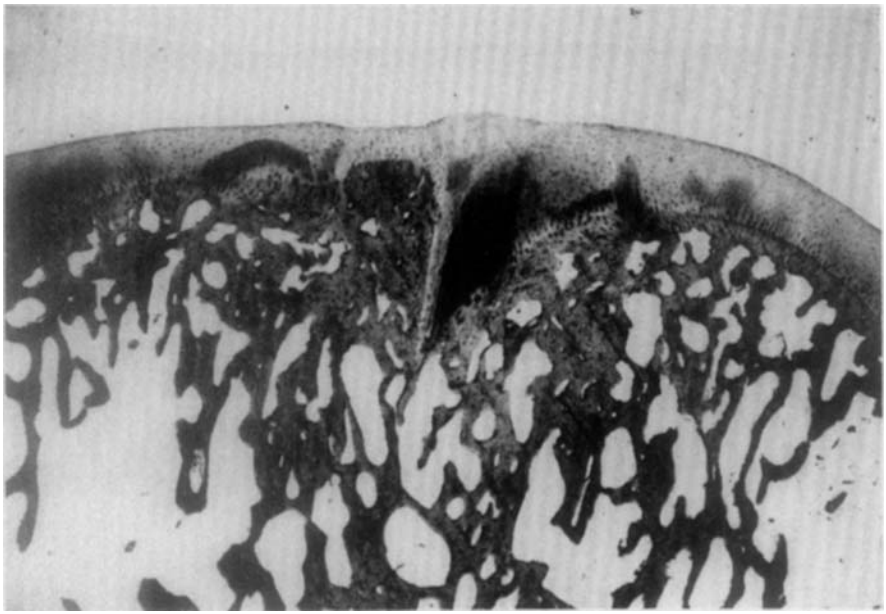


Figure 43. Histological sections from control defects in the joint surface of the femoral condyles filled with osseous transplants and devitalized cartilage (Tr.pl. D).
Observation period: 14 months.

A. General view of a defect.
(Haemalun-azophloxine-saffron, x 15)

The joint cartilage has grown down into the right half of the defect. To the left (corresponding to the slight depression of the joint surface) the process of ossification has extended somewhat beyond the basal level of the adjacent joint cartilage.



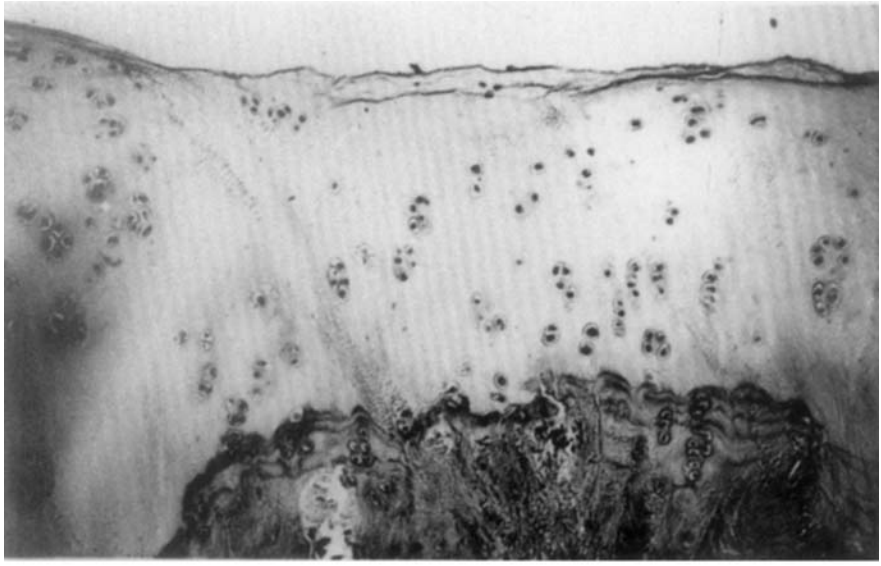
B. Detail from the left part of the defect seen in Figure 43 A. (Haemalun-azophloxine-saffron, x 150)

To the left: Joint cartilage. To the right: Cartilage of the central part of the defect. This cartilage also appears hyaline. A narrow basophilic zone is seen in the basal area.

C. General view of a defect. (Haemalun-azophloxine-saffron, x 15)

This picture demonstrates a defect in which the superficial part has been almost completely covered with cartilage which has obviously originated from the surrounding joint cartilage.

B



C