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Transplantation of Autologous Costal Cartilage to an Osteochondral Defect on the Femoral Head

Histological and Autoradiographical Studies in Adult Rabbits after Administration of ^{35}S-Sulphate and ^{3}H-Thymidine

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Transplantation von autologem Rippenknorpel in einen osteochondralen Defekt auf dem Hüftgelenkskopf

Eine histologische und autoradiographische Studie nach Zufuhr von ^{35}S-Sulfat und ^{3}H-Thymidine, ausgeführt an erwachsenen Kaninchen

Zusammenfassung. Am Hüftgelenkskopf von erwachsenen Kaninchen wurde ein $3 \times 4 \times 3$ mm großer osteochondraler Defekt geschaffen. In diesen wurden (A) autologer Rippenknorpel, welcher zuerst während 4—5 Wochen intramuskulär implantiert war (18 Tiere), und (B) frischer autologer Rippenknorpel (17 Tiere), transplantiert. An 7 verschiedenen Beobachtungszeitpunkten zwischen 1—46 Wochen wurden die Tiere getötet. Der Hüftgelenkskopf wurde in ^{35}S-Sulfat oder ^{3}H-Thymidine *in vitro* inkubiert und histologisch und autoradiografisch untersucht.

In 34 der 35 Präparate beider Versuchsgruppen und an allen Beobachtungszeitpunkten lag das Transplantationsgebiet im gleichen Niveau wie der übrige Gelenkknorpel.

Die histologische und autoradiographische Untersuchung zeigte eine Vereinigung des Transplantatknorpels mit dem Gelenkknorpel durch jungen, hyalinen Knorpel, mit hohem ^{35}S-Sulfat-Einbau, an allen Beobachtungszeiten von 4 Wochen aufwärts, in 13 der 14 Fälle der Gruppe A und in 12 der 13 Fälle in Gruppe B. Nach Versuchszeiten von 1 und 2 Wochen wurde junger, reifer Knorpel zwischen Transplantat und Gelenkknorpel in 2 der 4 Fälle der Gruppe A, aber in keinem der 4 Fälle in Gruppe B beobachtet. Unreifer Knorpel kam in 3 der 4 Fälle in Gruppe A und in 1 der 4 der Gruppe B vor.

Die Gelenkfläche des Transplantationsgebietes bestand zum überwiegenden Teil aus jungem, reifen Knorpel in 16 der 18 Fälle der Gruppe A (an allen Beobachtungszeitpunkten) und in 11 der 17 Fälle der Gruppe B (an allen Beobachtungszeiten von 4 Wochen aufwärts). Dieser junge Knorpel zeigte hohe ^{35}S-Sulfat-Aufnahme und enthielt ^{3}H-Thymidin-markierte Chondrocyten. In beiden Untersuchungsgruppen zeigten, nach Versuchszeiten von 8 Wochen aufwärts, die Chondrocyten dieses jungen Knorpels eine Tendenz, sich in Reihen senkrecht zur Gelenkfläche anzuordnen. Die Schichten nächst der Gelenkfläche zeigten eine mehr tangentiale Zellanordnung.

Die Transplantate trugen wahrscheinlich aktiv zur Wiederherstellung der Gelenkfläche des Defektgebietes und zur knorpeligen Vereinigung von Transplantat und Gelenkknorpel mit lebendem, hyalinem Knorpel bei. Auch örtliches Gewebe, aus dem subchondralen Knochengebiet, dürfte bis zu einem gewissen Teil zu dieser Wiederherstellung beigetragen haben.

Summary. (A) Autologous costal cartilage which had first been implanted intramuscularly for 4—5 weeks (18 animals) and (B) fresh autologous costal cartilage (17 animals) were transplanted to an osteochondral defect, measuring $3 \times 4 \times 3$ mm, on the femoral head in adult rabbits. After 7 different observation times between 1 and 46 weeks the animals were killed. The femoral head was incubated in ^{35}S-sulphate or ^{3}H-thymidine *in vitro* and examined histologically and autoradiographically.

In 34 of the 35 preparations from the two experimental groups, and at all observation times, the transplant area lay at a level with the remaining articular surface. Both histological and autoradiographical examination revealed union of the transplant with the articular cartilage via young hyaline cartilage, with a high ^{35}S-sulphate uptake, at observation times from 4 weeks onwards, in 13 out of 14 cases in group A and in 12 out of 13 cases in group B. At observation times of 1 and 2 weeks, young mature cartilage was found between the transplant and articular cartilage in 2 out of 4 cases in group A and in no cases in group B. Immature cartilage occurred in 3 out 4 cases in group A and in 1 out of 4 in group B.

The articular surface in the transplant area consisted to the greatest part of young mature cartilage in 16 of the 18 cases in group A (all observation times) and in 11 of the 17 cases in group B (at all observation times from 4 weeks onwards). This young cartilage showed a high ^{35}S-sulphate uptake and contained ^{3}H-thymidine labelled chondrocytes. At observation times from 8 weeks onwards the chondrocytes in this young cartilage in both experimental groups showed a tendency to a columnar arrangement oriented at right angles to the articular surface, and the layer of cells nearest to the articular surface were arranged tangentially to the joint cavity.

The transplants probably contributed actively to the cartilaginous union between the transplant and the articular cartilage and to restitution of the articular surface in the defect area with viable hyaline cartilage. Local tissue from the subchondral bone area also appeared to have contributed to some extent.

Traumatic defects in articular cartilage in adult mammals usually tend to be slow in healing. The articular cartilage itself usually offers only an insignificant quantitative contribution to such healing, and articular cartilage defects penetrating to the subchondral bone heal mainly by tissue arising from this area (for ref. see MANKIN, 1962; DE PALMA *et al.*, 1966; LEMPERG, 1967 d). In adult dogs and puppies, DE PALMA *et al.* (1966) found on histological examination that limited articular cartilage defects including the subchondral bone were filled after 16 weeks with tissue resembling hyaline cartilage. An autoradiographic examination, following intravenous administration of ^{3}H-thymidine and carried out on the same material, indicated that this hyaline cartilage probably arose from metaplasia of granulation tissue which had grown out from the subchondral bone. Numerous observations made in man and in commonly used experimental animals indicate, however, that this healing process is often incomplete depending, among other things, on the size of the defect, and does not lead with certainty to restitution of the articular surface with viable hyaline cartilage (HÄBLER, 1925; KEY, 1931; HALDEMAN, 1938; KETTUNEN, 1958; HOOVER and COVENTRY, 1961; CALANDRUCCIO and GILMER, 1962; LEMPERG *et al.*, 1965).

In attempts to replace damaged articular cartilage it has been found in animal experiments that the articular cartilage in viable autologous osteochondral articular cartilage transplants can survive for a long period without signs of pronounced degeneration (DE PALMA *et al.*, 1963). The articular cartilage in similar but homologous transplants has shown a more pronounced tendency to degeneration (DE PALMA *et al.*, 1963; CAMPBELL *et al.*, 1963). As regards such autologous transplants, these are hardly available in practice for treatment in man. Concerning preserved homologous osteochondral cartilage transplants and other, non-cartilage containing biological material, no convincing evidence has been provided that these could with certainty permanently restore a joint surface with living hyaline cartilage.

Fresh autologous costal cartilage has been transplanted to joints in man by WEGLOWSKY (1907), DUFOURMENTEL and DARCISSAC (1935), PADGETT *et al.* (1948) and LONGACRE and GILBY (1955) and good functional results have been reported. PEER (1955) and HIERTONN (1957) reported good functional results from autologous costal cartilage transplants which were first implanted intramuscularly and then transplanted further to the hip joint in man. YOUNG (1940), in experiments on dogs, and SIMONS and DANIS (1963), in the rabbit, transplanted viable autologous costal cartilage to an osteochondral defect in the knee joint and in the acetabulum of the hip joint, respectively. On a histological basis the transplants were considered to be viable after implantation periods of 12 and 5 months, respectively.

The present investigation was performed on the basis of observations in adult rabbits that viable autologous costal cartilage showed signs of new cartilage formation after about 4 weeks of intramuscular implantation. These observations were made in autoradiographic studies after labelling with ^{35}S-sulphate (LEMPERG, 1967a), chemical and radiochemical studies of incorporation of ^{35}S-sulphate in chondroitin sulphate (HJERTQUIST and LEMPERG, 1967) and autoradiographic studies after administration of ^{3}H-thymidine (LEMPERG, 1967c). It was observed, further, that such cartilage transplant also showed signs of new cartilage formation after free transplantation into the hip joint (LEMPERG, 1967b).

The aim of this investigation was to ascertain whether autologous costal cartilage, on transplantation to a limited osteochondral defect on the femoral head, could contribute to restitution of the articular surface with viable hyaline cartilage. Two experimental groups were studied: (A) autologous costal cartilage which was first implanted intramuscularly for 4—5 weeks and (B) fresh autologous costal cartilage.

Material and Methods

Thirty-five rabbits of both sexes, weighing 3,5—6,0 kg and with a given age of about, and no less than 1 year, were used for the experiments.

Transplantation Material

Group A. In 18 animals cartilage from the 6th and 7th ribs was cut into approximately 1 mm thick slices, placed between the walls of a double-walled perforated teflon cup and implanted intramuscularly for 4—5 weeks (LEMPERG, 1967a). After this period of implantation the pieces of cartilage were united by connective tissue. Part of this layer, in which the pieces of cartilage were macroscopically well united, was used for transplantation to the hip joint.

Group B. In 17 animals fresh costal cartilage was taken from the 6th or 7th rib and was used for direct transplantation to the hip joint.

Procedure for Hip Joint Operation

Under Nembutal anaesthesia, and sterile conditions, the hip joint was exposed from the dorsal side. The joint capsule was incised and the femoral head lifted out of the acetabulum as far as was allowed by the ligamentum teres without this being divided. On the dorsal articular surface, about 3 mm medial to the borderline between the neck and head of the femur, a defect with a surface area of about 3×4 mm and a depth of about 3 mm, and including the articular cartilage and the subchondral bone, was created with a dental drill. Using a fine scalpel, the articular cartilage adjacent to the margin of the defect was removed very carefully in order to obtain as even defect margins as possible. The transplant, consisting of 2—3 cartilage slices, fitted exactly into the defect. When the femoral head was replaced in the acetabulum the larger part of the transplantation area lay within the acetabulum and the transplant was

held in place without further fixation. The joint capsule and muscles were then sutured with fine catgut. No attempt at immobilization was made, and no antibiotics were given post-operatively.

Observation Times

After 1, 2, 4, 8, 12, 20 and 46 weeks the femoral head on both the operated and the intact side was removed after macroscopic inspection of the hip joints.

Incubation in ^{35}S-Sulphate[1] or ^{3}H-Thymidine[2] in vitro

At each observation time one or two femoral heads were incubated in ^{35}S-sulphate or ^{3}H-thymidine. The specimen was immersed in 2 ml sulphate-free Tyrode's solution in a water bath at 37° C under continuous agitation for 1 hour, as described previously (LEMPERG, 1967a). ^{35}S-sulphate was added in a quantity of 1 mC. The specimen was washed in saturated sodium sulphate solution after the incubation (HJERTQUIST and LEMPERG, 1967). ^{3}H-thymidine was added in a quantity of 10 μC; these specimens were washed in water (LEMPERG, 1967c). The effectiveness of the washing procedure for the removal of unbound radioactive material was studied in the investigations cited above.

Intraarticular Administration of ^{3}H-Thymidine

Two animals in each group, in all cases at an observation time of 4 weeks, were injected in the hip joint (MANKIN, 1962) with 10 μC ^{3}H-thymidine in 0,3 ml sterile isotonic NaCl solution 1 or 24 hours before death. The hip joint was first exposed surgically to ensure that the injection was intraarticular, and a very fine needle was used to prevent any reflux of injection solution.

Histology and Autoradiography

The specimens were fixed in 4% aqueous formaldehyde containing 0,5% cetylpyridinium chloride, decalcified in a mixture of equal parts of monosodium citrate (20%) and formic acid (44%) and embedded in paraffin. The specimens were then cut serially into 5 μ thick sections. The first 150 sections were used for autoradiography and every 10th following section for histology. Autoradiography was performed with the dipping technique (Ilford K 2 in gel form, diluted with distilled water 2:1) as described by KOPRIWA and LEBLOND (1962). The exposure time for ^{35}S-sulphate labelled specimens was 7 days and for those labelled with ^{3}H-thymidine 14 days. After development of the autoradiograms a number of the specimens were stained with hematoxylin and eosin or 1% toluidine blue in aqueous solution, while some remained unstained. For histological examination the specimens were stained by the same methods. Cells with more than 10 grains over the nucleus were considered to be labelled with ^{3}H-thymidine, indicating DNA synthesis (cf. LEMPERG, 1967c).

Results

Macroscopic Examination

With regard to wound healing, scar formation in the joint capsule, and the appearance of the synovial membrane, synovial fluid and articular cartilage in the acetabulum, no difference was found between the experimental groups and nothing noteworthy was observed. At 1 and 2 weeks the joint capsule was not healed with certainty. In no case did adhesions occur between the transplant area and the synovial membrane.

1. Carrier free ^{35}S-sulphate, SJSI, The Radiochemical Centre, Amersham, England.
2. Thymidine-methyl H^3, specific activity 3,0 C per mmol., Cat. No. 2532-95, Schwarz Bio Research Inc., Orangeburg, New York.

The transplant area on the femoral heads were visible in all cases owing to the somewhat lighter colour of the transplants (Fig. 1). In group A (previous intramuscular implantation) 16 of the 18 transplants were still *in situ*, while in 2 cases one piece of transplant had fallen out (2 and 12 weeks). In group B (fresh costal cartilage) 14 of the 17 transplants were still *in situ*, while in 3 cases (2, 4 and 8 weeks) the transplant had become partially detached. All transplants in group A were firmly attached in the defect. In group B the transplant was moveable in 4 cases (1, 4, 4 and 16 weeks). Total continuity between the transplant and the articular cartilage was observed in 13 cases in group A and in 7 cases in group B.

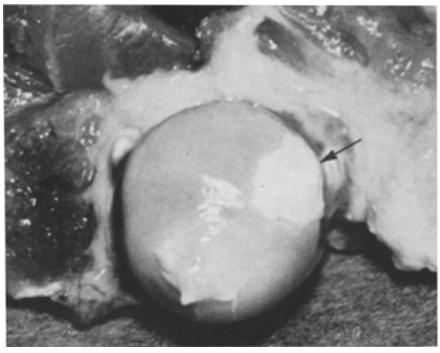

Fig. 1. Macrophotograph of femoral head showing the transplant (arrow) after an implantation period of 8 weeks. The transplant is clearly visible due to its lighter colour. It is in full contact with the surrounding articular cartilage and lies at the same level. Animal from group B

In 5 cases in group A (1, 2, 4, 12 and 12 weeks) and in 10 cases in group B (all 8 cases under 8 weeks and 1 case at 8 and 1 at 16 weeks) a *part* of the contact surface was not filled with tissue and a very narrow gap was visible. In all cases in both groups the transplant lay at a level with the articular surface (Fig. 1), except in one case (8 weeks) in group A, in which the transplant protruded 2—3 mm in the part of the transplant area lying outside the acetabular margin. The articular surface of the transplant area was usually even and smooth, but in some cases slightly uneven.

Histological and Autoradiographic Examination

Definition of Different Tissue and Cell Types in the Defect Area

In the defect area there were 5 main tissue components, and the names given below will be used for these in the following. This classification is limited by the fact that intermediate forms of these components often occurred, especially in the case of the less differentiated types.

a) Costal Cartilage (the transplant) was characterized by its special structure and cell arrangement (Fig. 2). It was denoted as viable when it exhibited ^{35}S labelling over the chondrocytes.

b) Young Mature Cartilage outside the margin of the costal cartilage consisted of cells of varying size and shape with a well-defined capsule, strongly stained nuclei and basophilic cytoplasm. The matrix was basophilic or metachromatic,

sometimes homogeneous and sometimes more fibrous. It exhibited a high ^{35}S uptake and at short observation times contained a small number of ^{3}H-thymidine-labelled chondrocytes. Morphologically it sometimes had some similarity to costal cartilage (Fig. 2) or articular cartilage (Figs. 3 and 4).

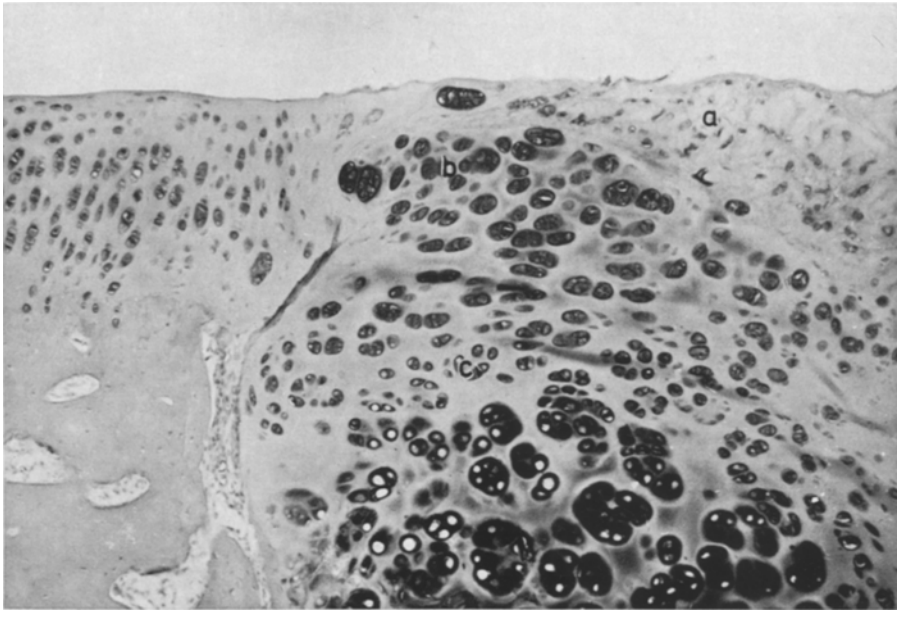

Fig. 2. Defect area after observation time of 4 weeks. The transplant lies at a level with the articular surface and is in full contact with the articular cartilage; part of the articular surface consists of immature cartilage (*a*). Contact between the transplant and articular cartilage has been established via young mature cartilage (*b*) with an appearance closely resembling that of costal cartilage (*c*). Animal from group B. Haematoxylin-Eosin. ×200

c) Immature Cartilage was rich in cells which were irregular in shape and had a well-defined capsule (Fig. 5). The intercellular substance was weakly basophilic or weakly metachromatic. It showed a distinct uptake of ^{35}S and contained a large number of ^{3}H-thymidine-labelled cells.

d) Mesenchymal Cells (KEMBER, 1960) had an oval or fusiform nucleus, weakly basophilic or unstained cytoplasm and weakly defined cell membrane (Fig. 6). This type of cell corresponds to the "osteoprogenitor cells" described by YOUNG (1962). This group also includes cells of the preosteoblast type (KEMBER, 1960) and the "prechondroblast" or "chondroblast" type (URIST *et al.*, 1965). They usually exhibited a low or no uptake of ^{35}S and contained a large number of ^{3}H-thymidine-labelled cells.

e) Fibrous Connective Tissue consisted of fusiform cells which showed ^{35}S labelling of varying intensity and various amounts of fibrous acidophil intercellular substance, and usually contained ^{3}H-thymidine-labelled cells. Perichondrial cells and "intermediate forms between cartilage cells and ordinary fibro-

blasts" (MAXIMOW and BLOOM, 1957) were difficult to distinguish from these cells and occurred frequently in the borderline areas between the fibrous connective tissue and the cartilage.

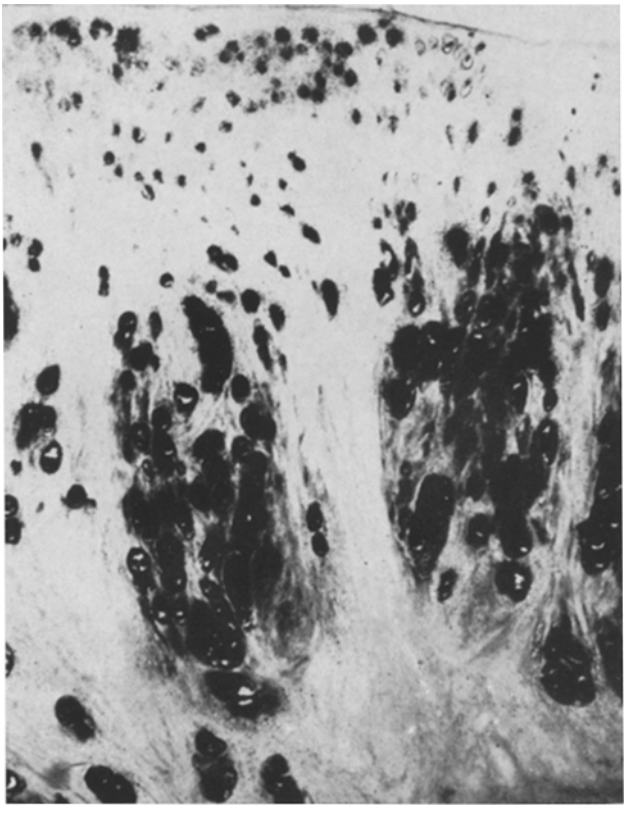

Fig. 3. Articular surface in the transplant area (area *b*, Fig. 7) after an observation period of 20 weeks. The chondrocytes in the deeper part of the transplant are arranged in columns oriented at right angles to the articular surface. Superficially the cells are oriented at a tangent to the joint cavity. Intensive uptake of ^{35}S. Animal from group A. Autoradiogram after ^{35}S-sulphate labelling weakly stained with Haematoxylin-Eosin. ×300

Observations in Different Areas

a) Contact Area between Transplant and Articular Cartilage (area *a*, Fig. 7). The observations concerning the type of tissue which united the transplant and articular cartilage are summarized in Table 1. In different parts of the contact area between the transplant and articular cartilage from the same specimen, different types of tissue could be observed. Signs of union of the transplant with the articular cartilage via young mature cartilage were observed within a small area in one of the specimens in group A after 1 week. At 2 weeks immature cartilage was observed in both specimens from group A (Fig. 5) and in one of them young mature cartilage which completely filled the space between the transplant and articular cartilage

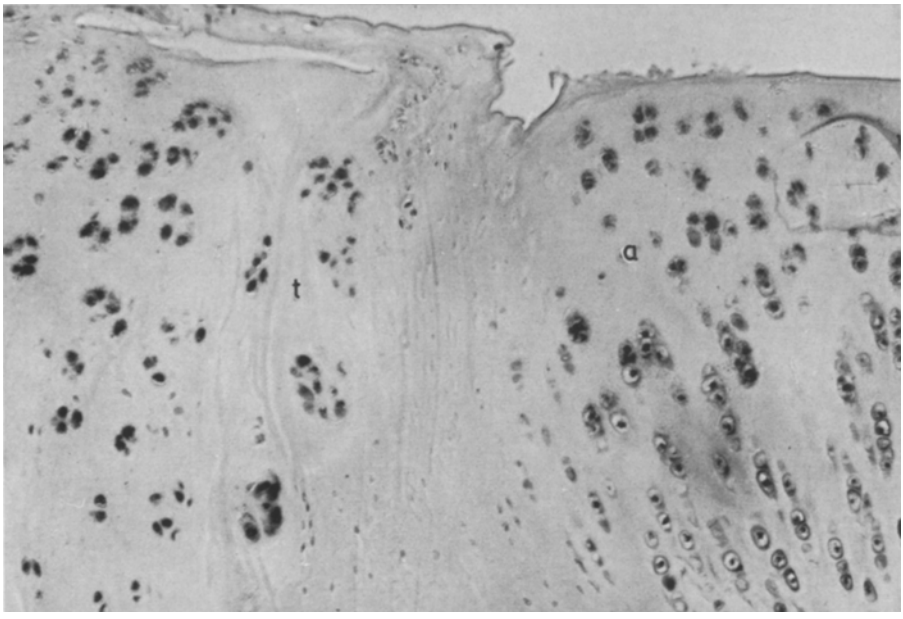

Fig. 4. On the borderline between the transplant area (*t*) and the articular cartilage (*a*) there is a zone of chondrocytes which show no uptake of ^{35}S-sulphate (area *e*, Fig. 7). This zone extends right out to the articular surface. The chondrocytes in the transplant area show an arrangement which has more similarity to articular than to costal cartilage. Animal from group A, observation time 16 weeks. Autoradiogram after ^{35}S-sulphate labelling weakly stained with Haematoxylin-Eosin. ×160

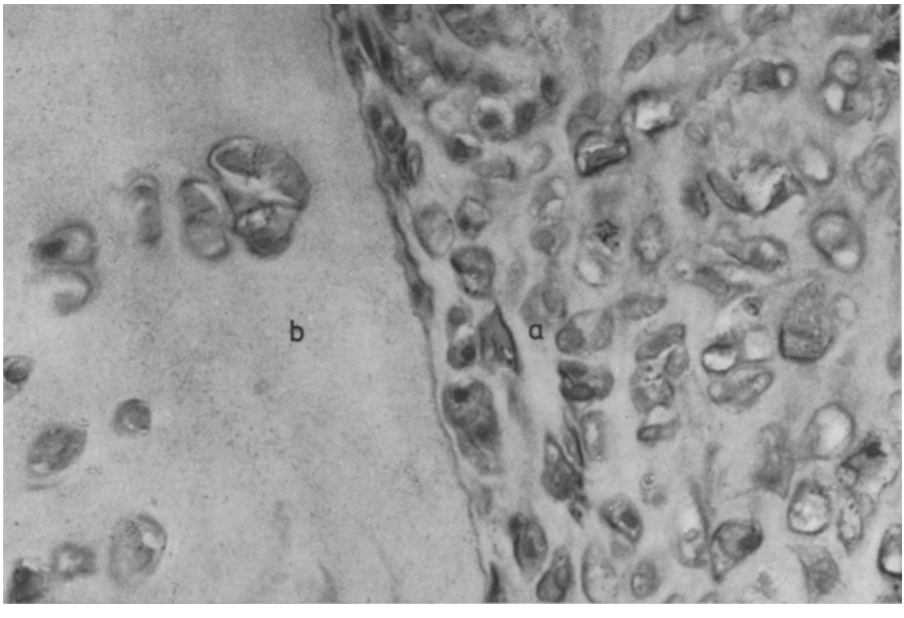

Fig. 5. Cell-rich tissue with the appearance of immature cartilage (*a*) close to the articular cartilage (*b*) which is poor in cells towards the margin of the defect. One ^{3}H-thymidine labelled cell in the centre. Observation time 2 weeks. Animal from group A. Autoradiogram, Haematoxylin-Eosin. ×700

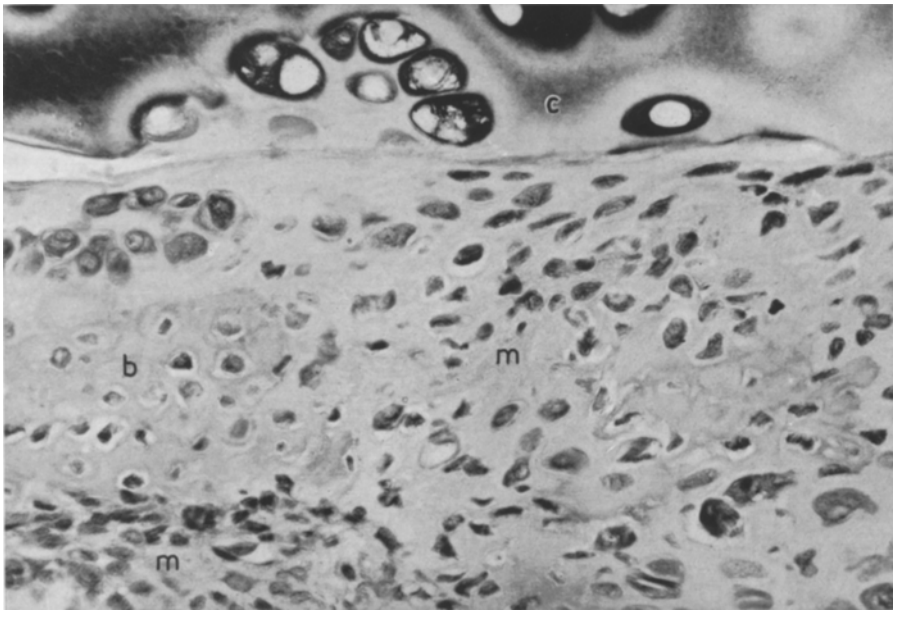

Fig. 6. Borderline between costal cartilage transplant (*c*) and osseous bed (area *c*, Fig. 7) after an observation time of 2 weeks (animal from group A). Cells of irregular shape with strongly stained cytoplasm and weakly defined cell membrane fill the central part of the picture; these cells are considered to be mesenchymal cells (*m*). At (*b*) new bone appears to have formed. Haematoxylin-Eosin. ×400

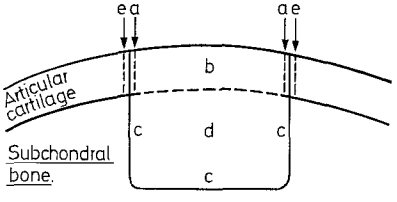

Fig. 7. Schematic diagram of the defect area with the adjacent articular cartilage. *a* contact surface between the transplant and articular cartilage. *b* articular surface in the defect area. *c* contact surface between transplant and osseous bed. *d* central part of the defect area. *e* articular cartilage in defect margin

was seen. In group B no cartilaginous tissue was observed in any of the specimens at 1 week, and at 2 weeks immature cartilage was seen in a small area in one case. Between the transplant and articular cartilage there were mainly mesenchymal cells lying close to one another, and connective tissue. At observation times of 4 weeks and upwards the space between the transplant and articular cartilage was filled with young mature cartilage in the majority of cases in both groups (Table 1 and Fig. 2). In some cases at short observation times this cartilage was similar in appearance to the costal cartilage (Fig. 2), while at longer observation times it usually showed some similarity to the articular cartilage (Figs. 3 and 4). In certain

Table 1. *Type of tissue occurring between transplant and articular cartilage (area a in Fig. 7)*

Group A: Costal cartilage which has been implanted intramuscularly for 4—5 weeks. Group B: Fresh costal cartilage. The figures represent the number of possitive findings. The definitions for the type of tissue are given in the text.

Observation time (weeks)	Group A				Group B			
	Number of animals	Fibrous connective tissue	Immature cartilage	Young mature cartilage	Number of animals	Fibrous connective tissue	Immature cartilage	Young mature cartilage
1	2	2	1	1	2	2	—	—
2	2	2	2	1	2	2	1	—
4	4	1	1	3	4	2	3	3
8	2	—	—	2	2	—	—	2
12	3	1	—	3	2	—	—	2
20	2	—	—	2	3	—	—	3
46	3	—	—	3	2	—	—	2

cases the young mature cartilage seemed to arise from the transplant. In other cases there was a continuous change-over from mesenchymal cells, arising from the subchondral bone area, to immature cartilage and young mature cartilage, indicating a continuous differentiation from mesenchymal cells to hyaline cartilage.

The observations on the *articular cartilage in the margin of the defect* (area *e*, Fig. 7) are reported for both experimental groups combined. In 10 cases and at all observation times the articular cartilage showed a zone of 2—4 cells which displayed no ^{35}S-labelling and in which the chondrocytes were probably dead (Fig. 4). Furthermore, in 19 cases and at all observation times the articular cartilage was poor in cells and showed poorly stained nuclei (Figs. 2, 4 and 5).

In all 10 specimens which had been labelled with ^{3}H-thymidine at observation times of 1—4 weeks, a few ^{3}H-thymidine labelled chondrocytes were found in all layers of the articular cartilage. These lay within the 5—10 rows of cells nearest to the margin of the defect, even beyond the zone of "dead" articular cartilage when such was present. In no case were labelled chondrocytes seen at observation times of 8 weeks and upwards. Sometimes there were chondrocytes in multinuclear chondrones, which showed labelling. These labelled chondrocytes did not, however, appear to contribute substantially to the cell population between the transplant and the articular cartilage. It was observed that even in cases where the articular cartilage was clearly dead, the transplant and articular cartilage had become united (Fig. 4).

b) Articular Surface in Defect Area (area *b*, Fig. 7). It is evident from Table 2 that different types of tissue occurred side by side in the same specimen. Young mature cartilage constituted the major part of the tissue in most specimens in which it occurred. In group A young mature cartilage was noted at shorter observation times than in group B. It appeared to have arisen mainly from the transplant, and this was best visible at short observation times (Fig. 2). The new cartilage usually lay on the costal cartilage in a layer facing the joint cavity (Fig. 2). In those cases where the costal cartilage lay directly at the joint cavity it constituted

Table 2. *Type of tissue occurring on the joints surface in defect area on the level with articular cartilage (area b in Fig. 7)*

Group A: Costal cartilage, which has been implanted intramuscularly for 4—5 weeks. Group B: Fresh costal cartilage. The figures represent the number of positive finding. The definitions for the type of tissues are given in the text.

Observation time (weeks)	Group A					Group B				
	Number of animals	Fibrous connective tissue	Immature cartilage	Young mature cartilage	Costal cartilage	Number of animals	Fibrous connective tissue	Immature cartilage	Young mature cartilage	Costal cartilage
1	2	2	1	1	2	2	2	—	—	2
2	2	1	2	2	1	2	2	1	—	2
4	4	2	4	4	1	4	2	3	2	3
8	2	2	2	2	—	2	1	2	2	—
12	3	2	1	2	1	2	—	1	2	—
20	2	—	1	2	—	3	—	—	3	—
46	3	—	—	3	1	2	—	—	2	2

only a small part of the articular surface and was viable in all cases. Costal cartilage constituting the articular surface of the defect area was observed in those cases where from the beginning it lay exactly at a level with the remaining articular surface, while young cartilage seemed to appear when the costal cartilage lay slightly below this level. In most cases and at all observation times the articular surface of the defect area lay at a level with the articular cartilage (Fig. 2).

At all observation times from 8 weeks onwards and in both experimental groups the chondrocytes in the young mature cartilage in the defect area showed a tendency to be arranged in columns oriented towards the articular surface (Figs. 3 and 4). The rows of cells nearest to the joint cavity were arranged tangentially to the articular surface (Figs. 3 and 4) with some similarity to the cell arrangement of the articular cartilage, and in most cases the articular surface was even. At an observation time of 46 weeks the articular surface in two cases was somewhat uneven, and in a few places frayed, multinuclear chondrones were seen there in large numbers. These changes were of the character of local cartilage degeneration. No bone was observed in any of the specimens.

c) Contact Surface between Transplant and Osseous Bed (area *c*, Fig. 7). The two experimental groups are reported in combination. At 1 week the greater part of the contact surface between the costal cartilage and the bone was filled with mesenchymal cells and connective tissue. At 2 and 4 weeks examination revealed mesenchymal cells, immature cartilage, young mature cartilage continuous with the costal cartilage and tissue having the appearance of newly formed bone which directly united the costal cartilage with the underlying bone (Fig. 8). The origin of the cartilaginous tissue seemed to be the same as reported above for area *a*, but in this case, differentiation from mesenchymal cells arising from the subchondral bone probably played a greater part than in area *a* (Fig. 6). At observation times between 8 and 20 weeks endochondral bone formation was found in the deepest parts of the costal cartilage but the bone-forming activity appeared to be

low. At 46 weeks incipient reconstruction of a subchondral bone plate was observed in some instances. The zone of calcified cartilage, characteristic for normal articular cartilage, was lacking, however, in most places.

d) Central Part of Defect Area (area *d*, Fig. 7). At observation times of up to 8 weeks this area consisted largely of costal cartilage. After 8 weeks the costal cartilage in the deeper parts had been replaced to varying extents by young

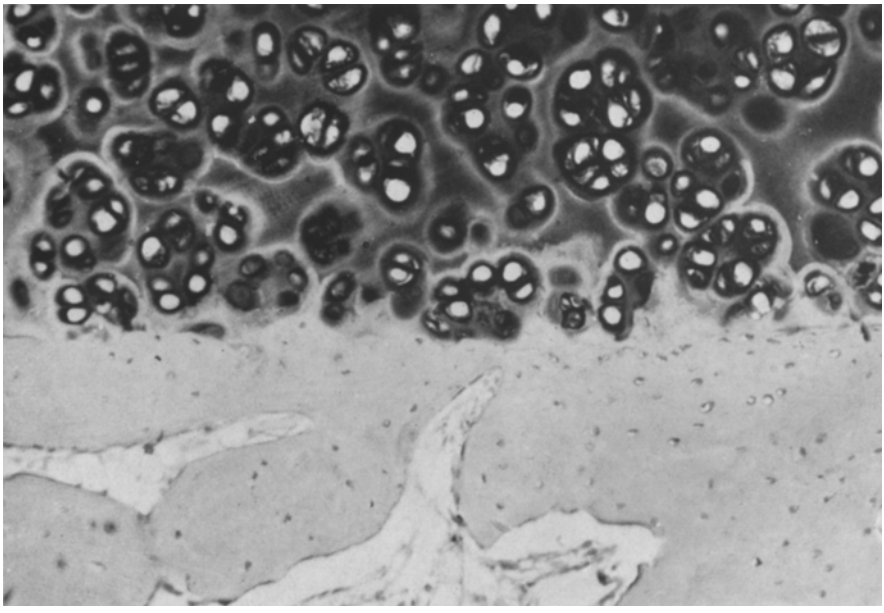

Fig. 8. Contact surface between transplant (costal cartilage) and base of defect. Note the intimate contact between bone and cartilage. The bone-forming activity is obviously low. Observation time 8 weeks. Animal from group A. Haematoxylin-Eosin. ×160

mature cartilage or by ingrown bone, which appeared mainly in such areas where the costal cartilage was dead. In group A mature young cartilage was observed after an observation time of 1 week. In group B such cartilage was seen from 4 weeks onwards. Fibrous connective tissue and occasionally mesenchymal cells were seen in very small amounts at all observation times.

e) Other Observations. In the ^{3}H-thymidine labelled specimens an attempt was made to calculate the labelling index in different cell populations in a reproducible way, which would permit a comparison between different animals. This was found to give unreliable results, however, owing to difficulties in identifying certain types of cell and to greatly varying labelling frequencies in different parts of *the same* transplant.

In the 5 cases (observation times 1—12 weeks) where part of the transplants had fallen out, it was observed how local tissue *alone* was able to restitute the defect. At the longest observation times the defect was filled with fibrous connective tissue with signs of metaplasia to fibrous cartilage. This, however, in no way

resembled the hyaline cartilage which constituted the articular surface at comparable observation times in successful transplantations.

The observations on animals which had been given ^{3}H-thymidine intraarticularly one hour before death did not differ from those made after *in vitro* labelling with regard to type of labelled cells. In the specimens from animals which received ^{3}H-thymidine 24 hours before death, several labelled mitoses were found in chondrocytes in young cartilage and in other cells in the transplant area. No labelled mitoses were observed with certainty in chondrocytes of the articular cartilage. On the other hand, multinuclear chondrones containing two labelled nuclei were seen. These were also observed, however, one hour after labelling *in vivo* and in *vitro*.

Discussion

The working hypothesis for this investigation was that restitution of a defect in an articular surface and union of a transplant and articular cartilage by tissue having the properties of the articular cartilage itself could most probably take place by means of new formation of cartilaginous tissue arising from the transplant. We therefore chose to study cartilage transplants which at the time of transplantation already showed signs of new cartilage formation. This had been established for autologous costal cartilage which had been implanted intramuscularly for 4—5 weeks (HJERTQUIST and LEMPERG, 1967; LEMPERG, 1967c). As controls, fresh autologous costal cartilage was transplanted to similar defects.

The morphological examination was complemented by autoradiographic studies after incubation in ^{35}S-sulphate *in vitro* or ^{3}H-thymidine *in vivo* and *in vitro*. Uptake of ^{35}S-sulphate takes place in chondroitin sulphate in the cartilage, which has been shown by BOSTRÖM and MÅNSSON (1952) to be an active enzymatic function of living chondrocytes. This has also been confirmed on a material similar to that used in the present study (HJERTQUIST and LEMPERG, 1967). Incorporation of ^{3}H-thymidine in the nucleus of cells indicates DNA synthesis which can be visualized by autoradiography (TAYLOR *et al.*, 1957).

YOUNG (1940), in adult dogs, transplanted fresh autologous costal cartilage to an osteochondral defect in the knee joint. He observed "cartilaginous union" between two pieces of costal cartilage transplanted to the same defect, but there was no such union between the transplants and the articular cartilage. SIMONS and DANIS (1963) transplanted fresh, autologous costal cartilage to an osteochondral defect in the acetabulum in rabbits. These authors observed new cartilage formation around the transplants, but no restitution of the articular surface with hyaline cartilage, or cartilaginous continuity between transplant and articular cartilage were found at observation times comparable with those used in the present investigation. This was probably due to the fact that with the operation method which they used, no *intimate* contact was obtained between the transplant and articular cartilage.

Of the many changes which can be observed on transplantation of autologous costal cartilage to an osteochondral defect on the femoral head, we have chosen to discuss mainly two questions, which would seem to be of major importance: 1) in what way does union, if any, between the transplants and articular cartilage take place and 2) is the articular surface restored by living hyaline cartilage?

The continuity between the transplant and articular cartilage was established by a sequence of processes. At 1 week the space in group A (previously implanted intramuscularly) was filled with closely situated mesenchymal cells, fibrous connective tissue, immature cartilage and young mature cartilage, while in group B only mesenchymal cells and fibrous connective tissue were observed. At 2 weeks the picture in group A was essentially the same, while in group B immature cartilage was now added. At 4 weeks and upwards viable hyaline cartilage united the articular cartilage and the transplant in most cases. This observation supports the view that the transplant is the main active contributor to this *early* new formation of cartilage, since in group A the transplants probably already contained viable newly formed cartilage at the time of transplantation. Also supporting this view is the fact that at short observation times the cartilage in the transplant area also showed morphological resemblance to costal cartilage. It appeared, however, as if part of the cartilaginous tissue in the contact area may have had its origin in the subchondral bone via continuous differentiation from mesenchymal cells. In our own experiments on adult rabbits (LEMPERG, 1967 b), at such short observation times as 2 and 4 weeks no hyaline cartilage was ever observed in cases where an articular cartilage defect penetrated the subchondral bone without the presence of a transplant. Neither do there appear to be any reports of filling of similar defects with cartilaginous tissue in such a short time in the rabbit (KEY, 1931; HALDEMAN, 1938). DEPALMA *et al.* (1966) described the occurrence of "immature chondroid tissue" in standardised osteochondral defects in the knee joint in young and adult *dogs*, but not, however, until after 8 weeks, and hyaline cartilage was not observed until after 16 weeks.

CALANDRUCCIO and GILMER (1962) consider that cartilaginous proliferation from the superficial layer of the articular cartilage can in certain cases contribute to the healing of an osteochondral articular defect. In the present investigation it was found that young cartilage between the transplant and articular cartilage occurred even in those cases where a zone of dead chondrocytes was present in the articular cartilage in the margin of the defect. This finding opposes the possibility that the articular cartilage could have contributed substantially to the cell population between the transplant and articular cartilage (see also MANKIN, 1962; DEPALMA *et al.*, 1966).

The articular surface in the defect area (area *b*) lay at the same level as the remaining articular surface, in both groups and at all observation times. In most cases it consisted of young mature cartilage. In group A such cartilage was noted at shorter observation times than in group B. At observation times from 8 weeks onwards the chondrocytes in the transplant area showed a tendency to be arranged in columns oriented at right angles to the articular surface. Further, an arrangement of the superficial layer of cells at a tangent to the joint cavity was seen, resembling that in the articular cartilage. These areas consisted of young mature cartilage with a high ^{35}S-sulphate uptake.

The hyaline cartilage which constituted the articular surface was viable even at the longest observation time (46 weeks); at this time, however, minor signs of degenerative changes were seen in two cases. In no case, on the other hand, were there any signs of ossification in progress on the articular surface.

The morphological and autoradiographic findings thus showed that the cartilage in the transplant area had some resemblance to the articular cartilage. Further studies are now in progress with the aim of determining whether the chemical composition of this cartilage also resembles that of the articular cartilage (HJERTQUIST and LEMPERG).

It is evident from the above that the healing process in an osteochondral defect on the articular surface of the femoral head to which autologous costal cartilage has been transplanted is very complex. In this investigation it has been shown that cartilaginous union occurs between the transplant and the articular cartilage and that the articular surface in the defect area is restored by tissue having the appearance of viable hyaline cartilage. There is much to indicate that it is tissue arising from the transplant that makes the greatest active contribution to this healing process. Local tissue, probably arising from the subchondral bone area, also appears to contribute to an extent which is difficult to evaluate quantitatively.

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