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Osteochondral Grafts in Growing Rabbits

Histological, Autoradiographic (^{35}S-Sulphate), Microangiographic, Microradiographic and Fluorochromic Studies*

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Summary. In 60-day (group A) and 90-day (group B) old rabbits a standardized osteochondral graft was taken from the distal articular surface of the femur and replanted immediately. Five animals in each group were observed at 9 different times between 3 days and 6 months. On histological and autoradiographic (^{35}S-sulphate) examination the following were found: In group A there was no ^{35}S uptake in the deep layers of the articular cartilage between 3 days and 1 week; in most cases there was normal articular cartilage in the transplants at 2 weeks to 6 months. In group B later changes (3 weeks—6 months), affecting the greater part of the articular cartilage, were observed. These changes appeared to be irreversible and were found in about $^1/_3$ of the cases. The other $^2/_3$ showed completely normal articular cartilage.

Fluorescence-microscopic (after tetracycline administration), microradiographic and microangiographic (Indian ink) studies revealed the following: Revascularization of the subchondral bone took place after 3–5 days. The ossification process in the subchondral area was restored within 5–7 days. The osseous part of the transplants healed by primary bone union within 1–2 weeks. The revascularization took place more rapidly in group A.

At the longest observation times (8 weeks and 6 months) in both groups slight flattening of the transplant area was seen, probably as a result of slightly retarded growth of the bone within the transplant.

In growing animals the articular cartilage has a dual function. Its superficial layer forms a sliding surface, while its deep area comprises the growth zone of the epiphyseal bone nucleus (cf. Mankin, 1962; McKibbin and Holdsworth, 1967). A number of experimental studies have shown that the articular cartilage in autologous osteochondral grafts in adult animals survive transplantation (for references see De Palma *et al.*, 1963; Aichroth, 1971). This is compatible with the assumption that the articular cartilage obtains its nourishment via the synovial fluid (for references see Honner and Thompson, 1971). In growing animals, on the other hand, some evidence has been presented that part of the nutrition takes place from the subchondral vascular network (cf. McKibbin and Holdsworth, 1966; Honner and Thompson, 1971). With increasing age and decreasing growth the importance of the subchondral route in the nutrition of the articular cartilage seems to decline. No systematic study appears to have been made in which the survival of the articular cartilage in autologous osteochondral grafts has been investigated in growing animals of different ages. In view of the function of the articular cartilage as a growth zone, it is conceivable that damage to this cartilage that might result from a temporary interruption in the circulation may give rise to growth disturbances in the osseous area of the epiphysis. This is of interest for

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evaluation of the prognosis in intraarticular fracture with avascular articular surface bearing fragments in children.

In the present investigation standardized osteochondral grafts (intraarticular fracture with avascular fragment) were created in the knee joint in 60- and 90-day old rabbits. The viability of the chondrocytes was studied autoradiographically after labelling with ^{35}S *in vitro*. New bone formation was studied by fluorescence microscopy after tetracycline administration, and revascularization of the graft by microangiography. The aim was to study: a) the viability of the articular cartilage in relation to the revascularization of the graft, b) late degenerative changes in the articular cartilage, c) growth disturbances in the osseous area of the epiphysis, and d) late changes in the subchondral bone.

Material and Methods

Group A. Albino rabbits with a controlled age of 60 ± 2 days.

Group B. Albino rabbits with a controlled age of 90 ± 2 days. In each group 5 animals were observed after 3 and 5 days, 1, 2, 3, 4, 6 and 8 weeks and 6 months. A total of 108 rabbits were included in the material.

Operative Procedure on the Knee Joint

Under Nembutal anaesthesia and sterile conditions the knee joint was opened by a medial incision. (A lateral incision was used at first but was abandoned because of frequent luxation of the patella.) Using a thin rotating saw (blade thickness 0.12 mm) and under flushing with NaCl solution a wedge-shaped transplant was removed from the ventral articular surface of the femur about 3 mm distal to the epiphyseal plate (Fig. 1). A template was used in order to obtain exactly standardized wedges with a base 1.0 cm broad and a medial depth of 0.8 cm. The transplant was washed briefly in physiological saline to remove small loose bone fragments and was replaced immediately in the defect. The transplants always lay firmly in the defect and showed no difference in level from the rest of the articular surface. Great care was taken not to disturb the articular cartilage during the operation. The joint capsule was sutured with catgut. No attempt at immobilization was made and no antibiotics were given postoperatively.

Methods of Examination

Oxytetracycline was given intramuscularly in a dose of 25 mg/kg body weight 2 days before the animal was killed. The distal part of both the operated and the non-operated femur was divided in the sagittal plane. One half of the specimen was fixed and dehydrated in absolute alcohol and embedded in methyl methacrylate. Consecutive sagittal sections were sawn out, ground down to 70–100 μ and mounted in Permount. The other half was incubated for 1 hour in 2 ml Tyrode's solution containing 1 mC ^{35}S-sulphate (cf. Lemperg, 1967). They were fixed in a 4% aqueous formalin solution containing 0.5% cetyl pyridinium chloride, decalcified and embedded in Paraplast. Five μ thick serial sections were prepared; the first 100 were used for autoradiography and every following tenth for histological examination. The autoradiograms were prepared by the dipping technique as described previously (cf. Lemperg, 1967). After development the autoradiograms were stained with haematoxylin and eosin or 1% toluidine blue in aqueous solution; some preparations were left unstained, however. The histological preparations were stained in a similar manner. The preparations were studied in both ordinary and polarized light. The fluorescence was examined in a Zeiss fluorescence microscope with a combination of exciter filters BG 38 and UG 1 and a barrier filter 41. Microradiographic examination was carried out in a Philips PW 1010/30 apparatus at 15 kV, the preparations being in direct contact with Kodak spectroscopic plates 649-0. Microangiography with Indian ink (Pelican Indian Ink, diluted with isotonic NaCl 1:4), administered through a catheter in the aorta as described by Hulth and Olerud (1962), was performed on 2 animals in each group, at each observation time. The knee joint from one of

the angiographed animals was incubated in ^{35}S-sulphate after completion of the angiography and treated as described above. The other animal was frozen immediately to $-30°$ C. The knee joint was excised, thawed in absolute alcohol and divided into two halves. One half was used for histological examination and the other was embedded in methyl methacrylate.

Results

In 12 animals luxation of the patella occurred and in 2 an infection. In 4 animals the transplant had changed its position. These 18 animals were excluded from further evaluation as all of them showed arthrotic changes of different degrees. At each observation time there were 5 animals without complications available for examination.

Postoperative Course and Gross Observations

The animals were taken out of their cages daily and after 2–4 days were able to run unhindered.

Apart from the postoperative changes there were no alterations in the joint capsule, the synovial membrane or synovial fluid. In all cases the transplant lay at a level with the articular surface; its margins were visible in the articular cartilage even at the longest observation times. At 8 weeks and 6 months the articular surface of the transplant was slightly rough in a few cases. Small periosteal osteophytes on the medial side (at the incision) were present in occasional cases. No definite changes were seen on the articular cartilage outside the transplant. Slight general hypertrophy of the entire distal end of the femur was observed in most animals from the end of the 8th week. In about half of the animals in both groups the transplant area was somewhat flatter than the rest of the articular surface and did not completely follow its curvature.

Histological and Autoradiographic Observations

The transplant area was divided into different zones which are illustrated schematically in Fig. 1.

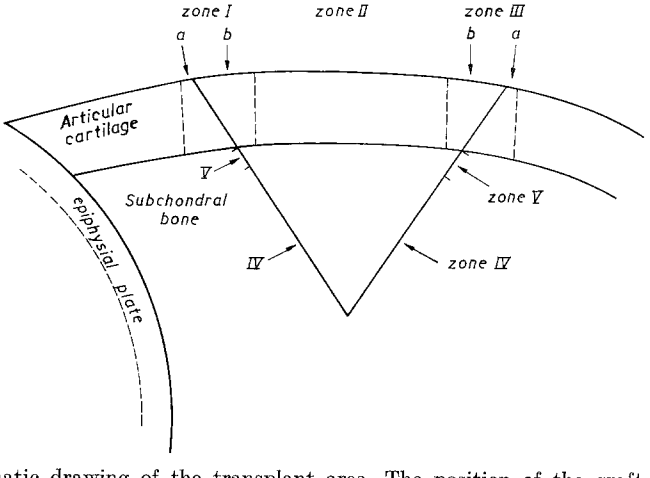
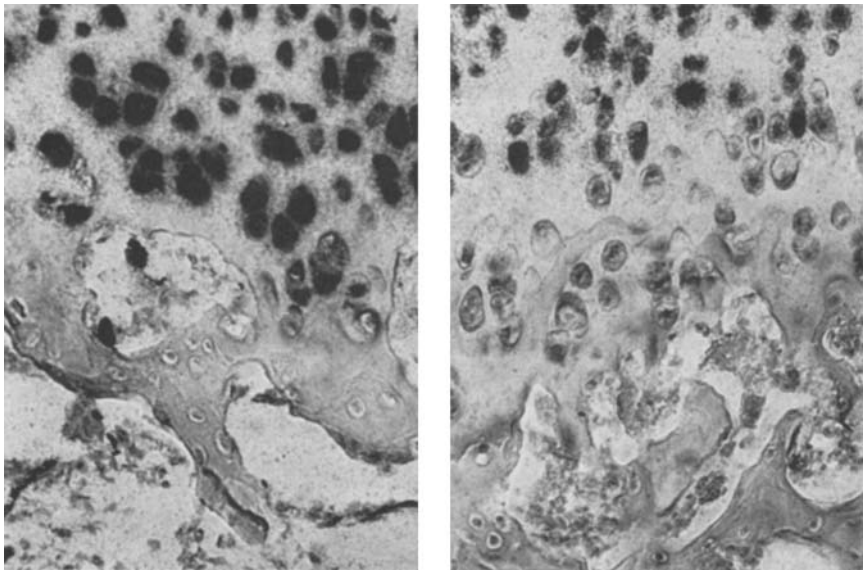


Fig. 1. Schematic drawing of the transplant area. The position of the graft distal to the epiphyseal plate is seen. Zone I (proximal) and zone III (distal) represent the articular cartilage adjacent to the incision surface and zone II the articular cartilage in the central part of the transplant. Zone V represent the osseous contact surface subchondrally and zone IV the remaining osseous bed



a b

Fig. 2a and b. Autoradiograms from an animal in group A angiographed with Indian ink at an observation time of 3 days. a, Normal articular cartilage outside the transplant; the chondrocytes adjacent to the bone show ^{35}S uptake. b, The articular cartilage in the transplant; the rows of chondrocytes nearest the bone show no ^{35}S uptake. In the subchondral medullary spaces poorly stained cells and thrombosed vessels are seen. The osteocytes have stainable nuclei. Haematoxylin and eosin ×300

Table 1. Autoradiographic and histological observations on the central part of the articular cartilage (zone II) of the transplant

Group A: 60-day old animals. Group B: 90-day old animals. Four animals were studied at each observation time in each group. The figures in the columns indicate the number of animals showing the following:

1. No ^{35}S uptake over chondrocyte groups in the deep layer alone (Fig. 2).
2. Articular cartilage in the transplant thicker than the adjacent normal cartilage (Fig. 3).
3. Residual cartilage in subchondral bone (Fig. 4).
4. Patchy distribution of labelled chondrocytes (Fig. 5).

Observation at	Group A				Group B			
	1.	2.	3.	4.	1.	2.	3.	4.
3 days	4	3	—	—	—	1	—	—
5 days	2	3	—	—	—	—	—	—
1 week	2	4	2	—	—	—	—	—
2 weeks	1	4	2	—	—	1	—	1
3 weeks	—	—	1	—	1	—	1	1
4 weeks	—	1	2	—	—	—	—	2
6 weeks	—	—	2	1	—	—	—	2
8 weeks	—	—	—	1	—	—	—	1
6 months	—	—	1	1	—	—	—	2

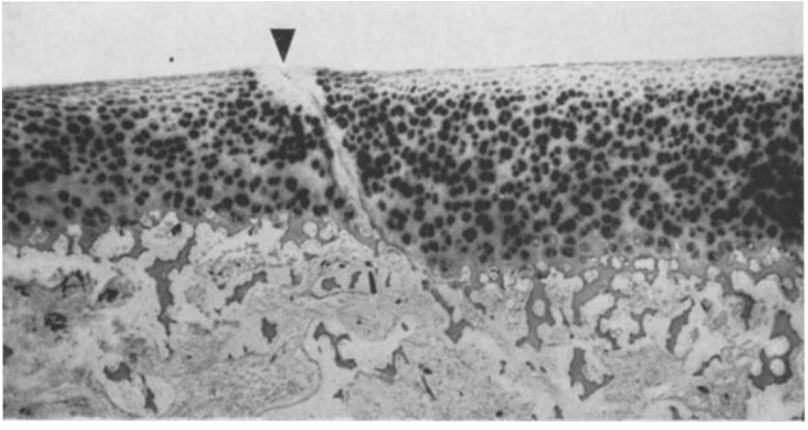


Fig. 3. Autoradiogram from an animal in group A at 1 week. The articular cartilage of the transplant (to the right) is thicker than the normal articular cartilage (to the left). Close to the incision surface (arrow) there are a few rows of unlabelled chondrocytes. Toluidine blue ×115

The Articular Cartilage in the Central Part of the Transplant (Zone II). In the majority of animals, especially in group B (90 days) the transplant cartilage was completely normal. As is evident from Table 1 early changes in the deepest layer of the articular cartilage in the form of unlabelled chondrocytes (Fig. 2) were common in group A (60-day animals). The chondrocytes in the deepest area of the epiphyseal plate showed uptake of ^{35}S. The articular cartilage of the transplant was thicker than the normal articular cartilage (Fig. 3) in the majority of animals in group A up to 2 weeks. In no case was the articular cartilage of the transplant thinner than the adjacent articular cartilage. Residual unossified articular cartilage, usually not labelled with ^{35}S, in the subchondral bone (Fig. 4), was common in group A. Late changes of a regressive nature were seen in the form of a patchy distribution of labelled chondrocytes (Fig. 5). In addition, at 6 months superficial fibrillation of the articular surface but without loss of substance was observed in 2 animals in each group. In occasional cases (see below) the transplant lay a few cell rows below the level of the articular surface, but this finding was not associated with any of the changes described above.

The Articular Cartilage Adjacent to the Incision Surface (Zones I and III). In 42 cases in group A and 41 cases in group B the transplants lay at a level with or less than 5 cell rows below the rest of the articular surface. A few rows of chondrocytes without ^{35}S uptake were observed close to the incision surface (Fig. 3) in about half of all cases. Intimate contact between the incision surfaces of the articular cartilage but with no bridging tissue in between was found in about two thirds of all cases at all observation times (Fig. 6). In the remaining cases the gap was filled with mesenchymal cells and immature cartilage up to an observation time of 4 weeks and thereafter mainly by hyaline cartilage. In a total of 7 cases where the articular cartilage of the transplant lay more than about five cell rows below the normal articular cartilage, fibrous tissue grew from the subchondral bone over adjacent articular cartilage.

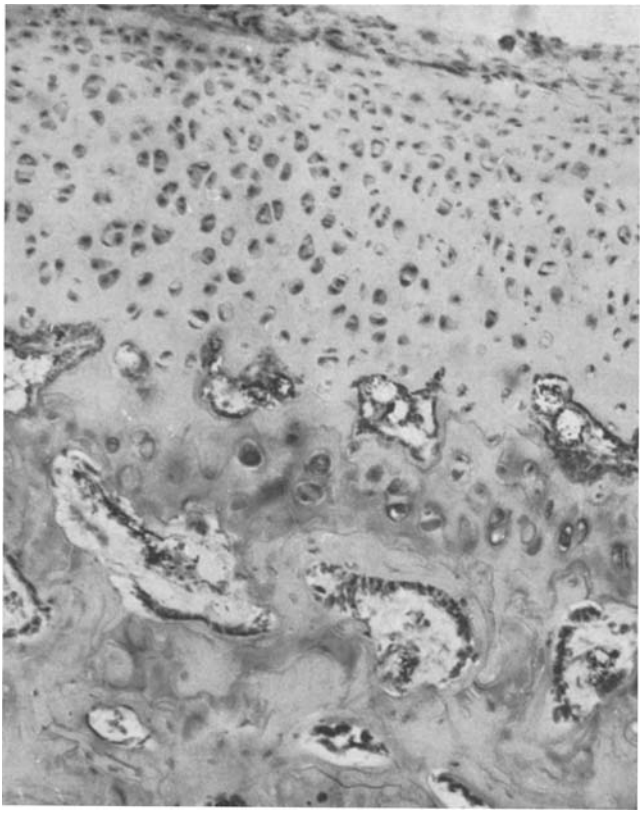


Fig. 4. Photomicrograph from an animal in group A after 2 weeks. Cartilaginous tissue (which on the autoradiograms shows no ^{35}S uptake) is seen in the subchondral bone. Hamatoxylin-eosin $\times 260$

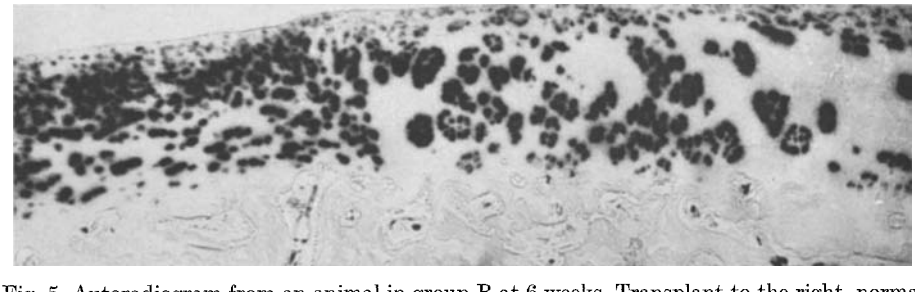


Fig. 5. Autoradiogram from an animal in group B at 6 weeks. Transplant to the right, normal articular cartilage to the left. Fewer chondrocytes with ^{35}S uptake are seen in the articular cartilage of the transplant. Multinuclear chondrones are frequent. Unstained preparation. $\times 100$

The Articular Cartilage Outside the Transplant showed in occasional cases limited superficial areas with no ^{35}S uptake (probably due to direct trauma) but otherwise no pathological changes.

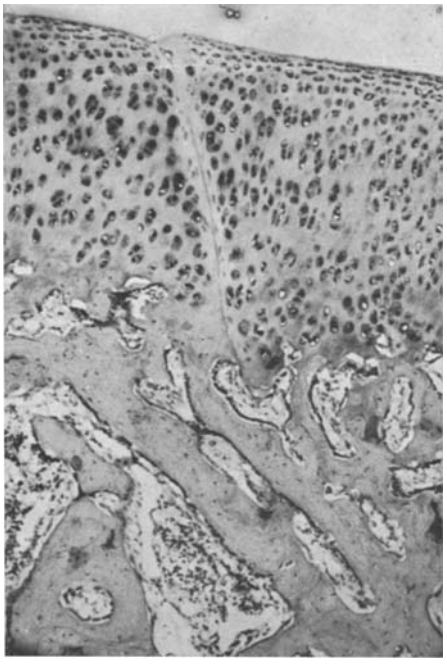
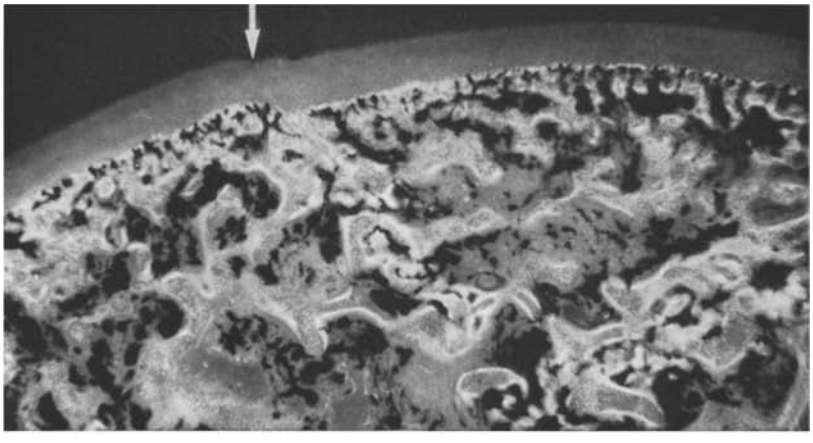


Fig. 6. Photomicrograph from an animal in group A after 2 weeks. Transplant to the right, normal articular cartilage to the left. There is intimate contact between the articular cartilage surfaces; in polarized light it is seen that there are no bridging collagen fibres in the fine gap between the transplant and articular cartilage. The subchondral bone shows complete healing via lamellar bone tissue. No callus tissue is seen. Haematoxylin and eosin. ×180

The Osseous Bed of the Transplant (Zones IV and V). Staining of the nuclei was reduced or absent in osteocytes and the cells in the medullary cavity in the greater part of the transplant at 3 days. The changes were most pronounced in the subchondral bone. The juxtachondral capillaries corresponding to zone II were thrombosed to a large extent (Fig. 2b). The changes were essentially similar in both experimental groups but distinctly more pronounced in group A. In zone IV primary bone healing was observed in practically all cases (Fig. 6) after 1 week. Cartilage callus was observed exceptionally in small areas up to a time point of 2 weeks.

Fluorescence Microscopic, Microradiographic and Microangiographic Observations

Subchondral Bone in Zone II. Table 2 presents the observations on the subchondral bone during the first two weeks. The tetracycline was administered 2 days before death, and the fluorescence observed on days 3 and 5 therefore reflects mainly the circulatory situation on days 1 and 3 (Fig. 7). It is evident from the table that the revascularization of zone II took place more rapidly in group A than in group B but that in 2 animals in group A at 1 week there was no fluorescence subchondrally. The subchondral vessels were clearly dilated in most cases in group A. At 2 weeks the fluorescence in group A was usually more extensive in the subchondral capillaries than outside the transplant. In group B it



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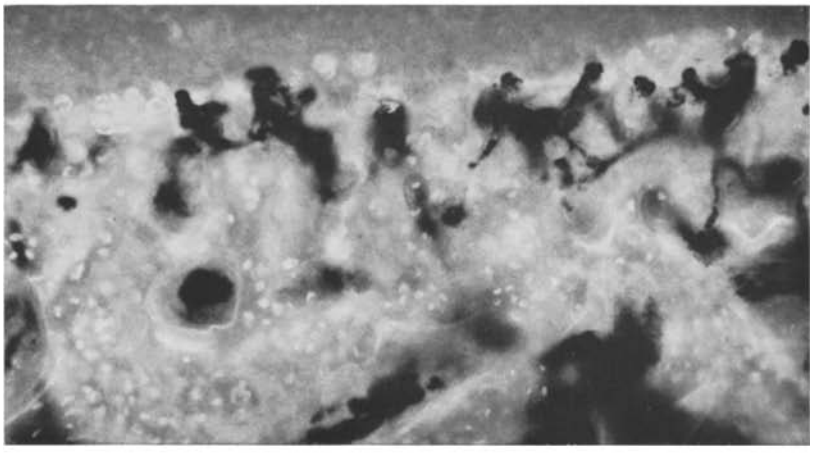

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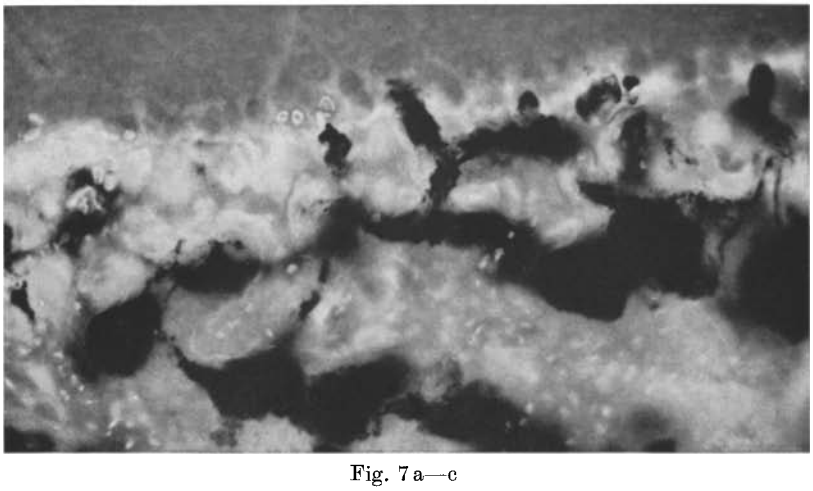


c

Fig. 7 a—c

Table 2. Observations within the two first weeks on the subchondral bone of the central part of the transplant (zone II) on fluorescence-microscopic and angiographic examination

At each observation 5 animals were examined by fluorescencemicroscopy and 2 by microangiography. Tetracycline was administered two days prior to death. Group A: 60-day animals; Group B 90-day animals.

1. Fluorescence in subchondral capillaries (Fig. 8a).
2. Fluorescence on lamellar bone surfaces (Fig. 8b).
3. Indian Ink filling of subchondral capillaries (Fig. 7).
4. Filling of larger subchondral vessels (Fig. 7).

The figures in the columns indicate the number of animals showing positive findings.

Observation at	Group A		Group B		Group A		Group B	
	1.	2.	1.	2.	3.	4.	3.	4.
3 days	—	—	—	—	1[a]	2[a]	—	2[b]
5 days	3	5	—	1	2[a]	2[a]	1[b]	2[b]
1 week	3	4	5	4	2[a]	2[a]	2[b]	2
2 weeks	5	5	5	5	2	2	2	2[a]

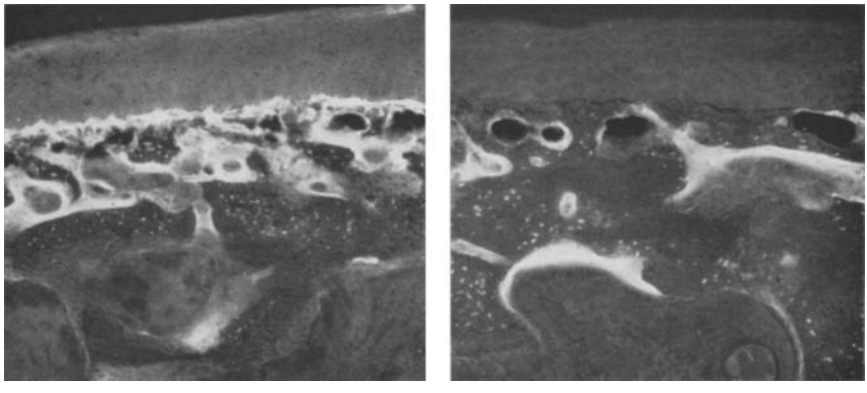
[a] The vessels showed dilatation (Fig. 7).
[b] Incomplete filling.

was mainly localized to lamellar bone surfaces, while it was reduced in the capillaries (Fig. 8 and 8b).

From the end of the 3rd week the ossification process had become normal in most animals. However, disturbed ossification of apparently two different types was observed. In the one type—seen in 3 cases in group A and in 6 in group B, degenerative changes were found in the articular cartilage. The fluorescence in the subchondral capillaries was increased or persisted, and in most cases the bone-cartilage borderline lay deeper than in the normal articular cartilage (Fig. 9). In the other type (3 cases in each group) no changes of the articular cartilage were observed. The fluorescence in the subchondral capillaries was weak or absent (Fig. 10). In 2 of these cases microangiograms were available and in both cases the subchondral vessels were incompletely filled in these areas. In 2 cases degenerative changes in the articular cartilage were not accompanied by any visible reaction in the subchondral bone.

The Ososeus Bed of the Transplant (Zones IV and V). On day 5 in group A and on day 7 in group B mineralized bone tissue was seen on the microradiograms and after 2 weeks in all cases fully mineralized cancellous bone bridged the gap between the transplant and osseous bed. In zone V cartilage was found between the transplant and osseous bed at observation times between 3 and 8 weeks in 11 cases in group A and 10 cases in group B (Fig. 9).

Fig. 7a—c. Fluorescence photomicrographs from an animal in group A at 5 days; angiography with Indian ink. a, Normal articular surface to the left of the arrow, transplant to the right. The greater part of the transplant is revascularized and the larger subchondral vessels seem to be dilated. Fluorescence is seen on a large number of bone surfaces in the transplant, and active fluorescence in the transplant bed. ×40. b, Detail from (a) Bone-cartilage borderline in normal articular cartilage with well filled capillaries. ×260. c, Detail from (a) Bone-cartilage borderline in the transplant; not all capillaries are filled, and some subchondral vessels are dilated. ×260



a b

Fig. 8a and b. Fluorescence photomicrographs from an animal in group B at 2 weeks. a, Normal articular surface. b, Articular surface in zone II of the transplant, where the major part of the fluorescence is localized to lamellar bone surfaces; only a few subchondral capillaries shows fluorescence and there is no fluorescence at the bone-cartilage borderline. ×60

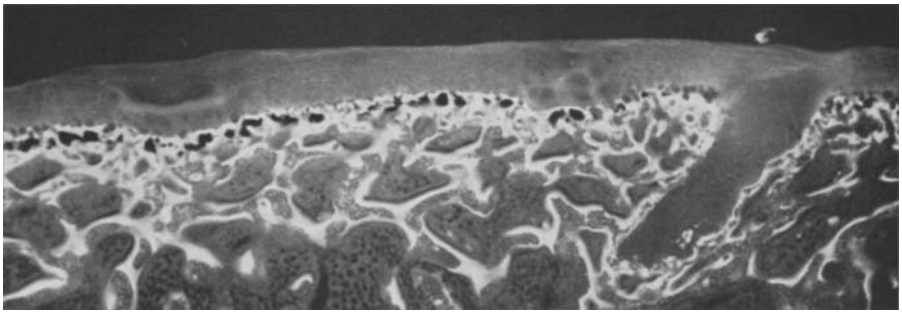


Fig. 9. Fluorescence photomicrograph from an animal in group A at 4 weeks. Degenerative changes are seen in two places in the articular cartilage of the transplant. The ossification there is retarded but there is fluorescence subchondrally. Between the transplant (left) and the osseous bed (right) there is a tongue of cartilaginous tissue extending down into the epiphyseal bone. ×35

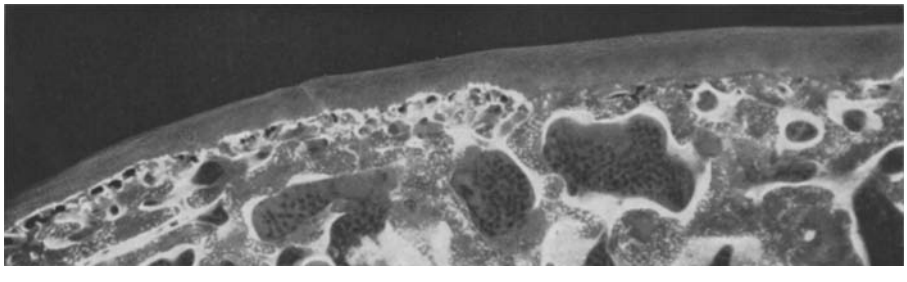


Fig. 10. Fluorescence microgram from an animal in group B at 6 weeks. There is weak fluorescence in the major part of the subchondral bone in the transplant (to the right); the articular cartilage shows no visible changes but is somewhat thicker in the transplant. In some parts of the transplant the bone-cartilage borderline lies at a lower level than the normal borderline. ×35

The Central Part of the Transplant. Fluorescence was visible on most bone surfaces in the central parts of the transplant on day 5 in both groups. At the same time the vascular system in the medullary cavity was well filled with Indian ink. The fluorescence was very active up to 2–3 weeks, after which it returned to normal.

Discussion

With this experimental model an attempt was made to study the effects of temporary interruption of the subchondral circulation in growing rabbits of different ages in order to determine the importance of this factor for the viability and growth of the articular cartilage. The experimental conditions were rigidly standardized, they gave optimal prerequisites for healing and excluded to the greatest possible extent other factors known to interfere with the integrity of the articular cartilage, such as immobilization, incongruence of the articular surface, permanent pressure, haemarthrosis or direct trauma (cf. Trueta, 1968).

The revascularization of the transplant took place more rapidly than that described by Stringa (1957). The observations on the osseous part of the transplant do not differ essentially from those made and discussed by De Palma *et al.* (1963), among others.

In the majority of cases congruence of the articular surface and full viability of the articular cartilage were observed. In about half of the cases at observation times after 8 weeks the articular surface of the transplant was, however, somewhat flatter, probably due to some retardation of growth. An absolutely exact position of the transplant appeared to be necessary for an optimal result. Even very slight lowering of the articular surface of the transplant, invisible with the naked eye, led to a growth of fibrous tissue over the articular cartilage.

The relationship between the temporary interruption of the subchondral circulation and the autoradiographic observations on the articular cartilage illustrates the importance of subchondral nutrition for the viability of the articular cartilage at different ages. In the 60-day old animals (group A), changes clearly localized to the deepest layer were found at 3 and 5 days, while in the 90-day old animals (group B) no such changes were observed. This may mean that in the younger animals the chondrocytes in the deepest layer are completely dependent upon the subchondral vessels for their nutrition.

When the subchondral circulation in group A had been restored, these unlabelled rows of chondrocytes were gradually resorbed, but clearly at a slower rate and with more irregularity than normally. These observations are in agreement with those of McKibbin and Holdsworth (1966) in 6-week old rabbits. The conclusion arrived at by these authors that undisturbed ossification of chondrocytes is an active process of maturation rather than a degenerative process also gains support from the present results.

McKibbin and Holdsworth (1966) also conclused that the growth of the articular cartilage was inhibited by interruption of the subchondral circulation. In the majority of cases in group A the thickness of the articular cartilage increased, however, in the period when the circulation was cut off. On comparison of the ^{35}S autoradiograms of the present study with the ^{3}H-thymidine autoradiograms in the publication of Mankin (1962) it is evident that the zones that

showed ^{3}H-thymidine incorporation also as a rule showed ^{35}S labelling and therefore were probably not affected by the interruption in circulation. This would mean that diffusion from the synovial fluid, at least temporarily, can provide sufficient nutrition even for the growth of articular cartilage.

A noteworthy observation was the patchy distribution of labelled chondrocytes in all layers of the articular cartilage, except in the superficial layer, at the longer observation times and mainly in group B. This change meant that the number of viable chondrocytes per volume was reduced, and must be regarded as degenerative; it showed no tendency to regression. Whether or not this led to further degeneration of the articular cartilage could not be determined in the present investigation. No arthrotic changes with subchondral osteophytes were observed with certainty in any of the cases.

The superficial degeneration of the articular cartilage, observed at 6 months, was not associated with any of the other previously discussed findings. It was possibly a consequence of slight incongruence of the articular surface.

The conclusion that can be drawn from this study is that the articular cartilage on avascular articular surface bearing fragments that have been replaced in an exact position essentially retains its viability and continues to grow. In the younger animals the picture was dominated by early ossification disturbances in the subchondral bone, which were reversible. In the older animals later changes of a regressive nature in the articular cartilage predominated and were probably irreversible.

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