Acta orthop. scand. 46, 11–24, 1975

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AUTOGENOUS TRANSPLANTATION OF APOPHYSEAL CARTILAGE TO OSTEOCHONDRAL DEFECTS OF JOINTS

An Evaluation of the Vitality by Means of Autoradiography (^{35}S)

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Accepted 21.x.74

An experimental investigation has been performed, using dogs, to determine the basic histological changes that take place in cartilage of traction epiphyses (apophyseal cartilage) following autogenous transplantation to osteochondral defects of joints, whereupon it is exposed to the same types of mechanical stimuli as the joint cartilage of pressure epiphyses. The macroscopical and morphological findings have been reported elsewhere (Benum 1974). Morphologically the cartilage appeared to remain vital following the transplantation, except for some parts of the growth plate overlying the metaphyseal osseous part of the transplants and the central basal regions of the overlying cartilage. These areas underwent necrosis to varying extents. The necrotic regions never extended into the cartilage superficially to the base level of the surrounding joint cartilage. This cartilage was obviously sufficiently nourished by the synovial fluid. Furthermore, the mechanical stimuli of joint function were found to prevent the ossification of the most superficial zone and contributed to the formation of a persisting and apparently vital cartilage that resembled true articular cartilage.

The purpose of the autoradiographic study, which will be presented here, was to determine if the cartilage was also able to synthetize sulphur-containing metabolites following injection of ^{35}S labelled sulphate. The application of this method as a functional test of the vitality of the transplanted cartilage also necessitated a study of the normal pattern of distribution of ^{35}S within apophyseal cartilage.

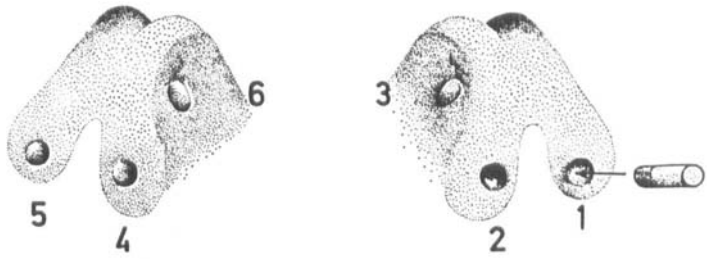

Figure 1. The picture shows the position of the defects in both knees. Defects nos. 1 and 4 were filled with osseous transplants and devitalized cartilage or partially filled with pure osseous transplants. Osteochondral transplants were implanted in the defects nos. 2 and 5, and also in the defects nos. 3 and 6, outside the cartilaginous joint surface.

MATERIALS AND METHODS

Twenty of the 24 puppies used in the morphological study were used in the present investigation. The details concerning the material, operative procedure and preparation of specimens for the histological investigations have been presented elsewhere (Benum 1974). Here it should suffice to summarize that 4 mm wide cylindrical transplants consisting of apophyseal cartilage and adjacent metaphyseal bone, removed from the iliac crest in 3- to 4-month-old dogs before a secondary centre of ossification had appeared within the iliac crest, were implanted into osteochondral defects of the femoral condyles. In each animal one osteochondral fragment was implanted into a weightbearing area of the medial condyle of the left knee and one into a corresponding area of the lateral condyle of the right. In 12 of the dogs similar transplants were implanted into a similar defect of the opposite femoral condyle of both knees, but first after devitilization of the cartilage by heating at 50° C in sterile Ringers solution for 30 minutes. Corresponding defects of the remaining 12 animals were partially filled with pure osseous transplants. Finally one osteochondral fragment was implanted into a defect outside the cartilaginous joint surface of the medial femoral condyle of both knees in all the puppies. The locations of the defects and the various transplants are shown in Figure 1. The animals were sacrificed at the following observation times; 2, 6 and 12 weeks, 6, 9 and 14 months giving the following numbers of transplants at each observation period: eight osteochondral weightbearing transplants, four weightbearing devitalized cartilage transplants, four pure osseous transplants in weightbearing defects and eight non-weightbearing osteochondral transplants. Both at 6 weeks and 9 months, however, the transplants of only two of the four animals could be prepared for autoradiography, for technical reasons, giving only half the number of transplants available for autoradiography within the various groups at these observation times. The pure osseous transplants were not studied autoradiographically.

48 hours prior to sacrifice the animals were injected intravenously with 2 milliCi ^{35}S/kg body weight as $Na_2{}^{35}SO_4$ diluted in 0.9 per cent NaCl. The resected blocks containing the transplants and the specimens from the iliac crest were washed with

0.9 per cent NaCl to remove synovial fluid or blood containing radioactively labelled sulphate, fixed for 24 hours in 4 per cent formaldehyde solution buffered with hexamethylene-tetramin, pH 7.4, decalcified with 7 per cent nitric acid for another 24 hours and then washed with water, treated in ethanol-xylene and finally embedded in paraffin. 5-micron thick sections were cut from the same regions as the sections which were to be studied morphologically. Following removal of the paraffin with xylene the mounted sections were passed down through ethanol to water. Uncoloured sections were used for autoradiographic investigations, applying the stripping-film technique. Kodak A.R. 10 was used and the time of exposure was 6 weeks.

Following development of the autoradiographs which was performed in a Kodak D 19b developer at 18° C for 4.5 minutes, the autoradiographs were examined without staining. Autoradiographs of sections from the femoral condyles of animals that had not been given any isotope served as controls. No attempts were made to perform exact quantitative analyses of the concentration of the labelling. All autoradiographs that did not contain significant labelling corresponding to the joint cartilage surrounding the transplant were discarded.

EXPERIMENTAL RESULTS

Iliac crest

There was a marked concentration of granules corresponding to the cartilaginous portion of the iliac crest at all periods of observation. The concentration of labelled sulphur was particularly high in the growth plate corresponding to the proliferating and hypertrophying cell layers,

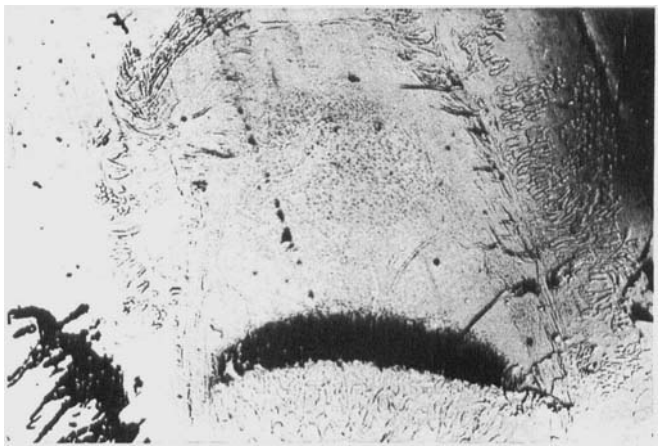

Figure 2. Autoradiograph of cross section from the middle part of the iliac crest of a $4^1/_2$-month-old dog (× 10). A secondary centre of ossification has not appeared within this part of the iliac crest. The labelling is particularly high corresponding to the position of the growth plate, but there is a marked labelling corresponding to the overlying cartilage also.

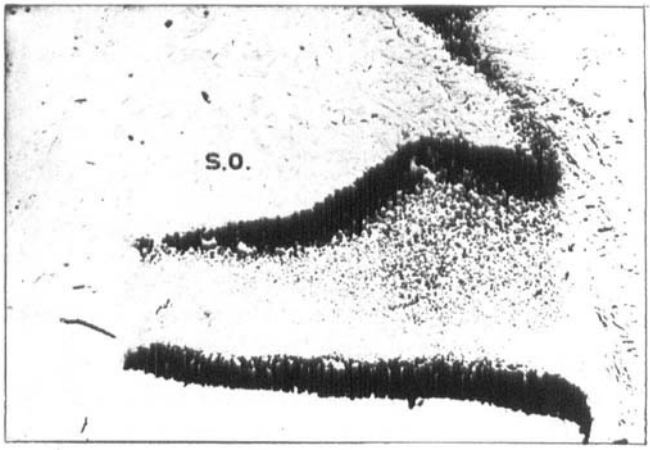

Figure 3 A. Autoradiograph of cross section from the posterior part of the same iliac crest as in Figure 2. A secondary centre of ossification has appeared within this part of the iliac crest (S.O.) (×10). The labelling is very high corresponding to the growth plate (below) and the cartilage near the secondary centre of ossification (S.O., above). There is also significant labelling over the cartilage between these areas.

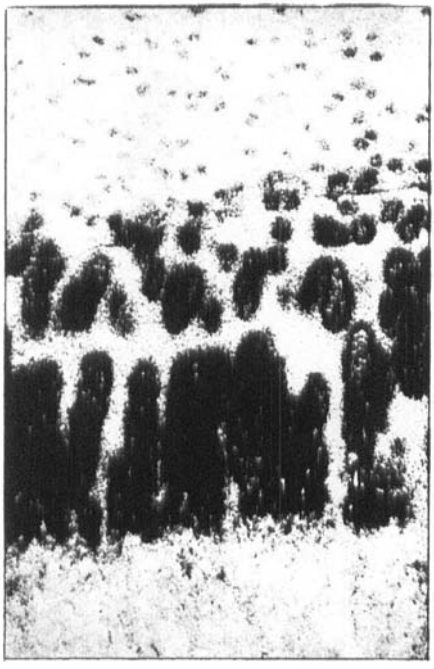

Figure 3 B. Detail from the growth plate shown in Figure 3 A (× 100). The labelling is concentrated over and around the cartilage cells of the growth plate, but there is also some labelling over the overlying cells and over the matrix.

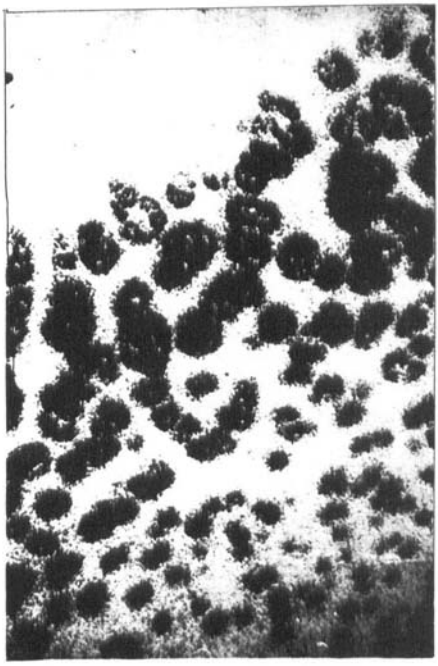

Figure 3 C. Detail from the cartilage adjacent to the secondary centre of ossification shown in Figure 3 A (× 100). The picture demonstrates a heavy labelling over and around the swollen cartilage cells (compare Figure 3 D) near the ossification centre. There is also some labelling corresponding to the matrix between the cells.

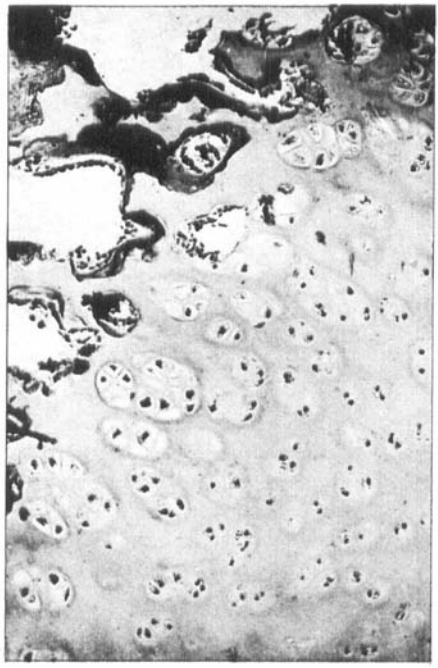

Figure 3 D. Morphological detail from the same area as in Figure 3 C (Haem-alun-azophloxine-saffron, × 100). The picture demonstrates markedly swollen cartilage cells near the ossification centre.

(Figures 2, 3, A and B, 4 A). In all regions of the apophysis the concentration of labelled sulphur was heaviest in and around the cartilage cells but even the matrix between the cells appeared to be labelled (Figures 3, B and C, 4 B). During the initial stage of the secondary ossification of any segment of the iliac crest, a definitely increased labelling was observed adjacent to the ossification centre corresponding to the regions where hypertrophic cartilage cells were demonstrated morphologically (Figures 3, A, C and D). As the ossicle increased in size and extended towards the growth plate, and as hypertrophic cartilage cells were no longer seen adjacent to the ossification centre by the morphological investigation, the cartilage in these regions no

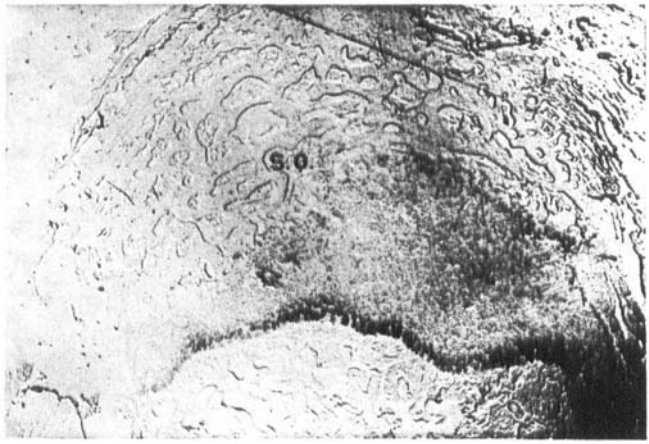

Figure 4 A. Autoradiograph of a cross section from the iliac crest of a 9½-month-old dog during the advanced stage of secondary ossification (× 10). The labelling over the cartilage adjacent to the centre of secondary ossification (s.o.) is not significantly higher than over the cartilage of the central areas. There is a heavy labelling corresponding to the growth plate.

longer seemed to contain larger amounts of labelled sulphur than the rest of the cartilage overlying the growth plate (Figure 4 A, B and C).

Osteochondral transplants to the joint surface

At all observation times the cartilage cells of regions which morphologically had appeared to be vital concentrated radioactively labelled sulphur. The occurrence of granules was particularly abundant over and around the cells. Corresponding to the regions which morphologically seemed to be necrotic, only a few granules were seen and there was no tendency to concentration of granules around the cells (Figure 5). Thus the central parts of the growth plates and possibly some central parts of the overlying cartilage did not contain labelled sulphur in the 2-week specimens.

At later observation times the unlabelled areas within the persisting unossified cartilage decreased in accordance with the reduction of the necrotic areas seen in the morphological study. The concentration of granules over and around the cells was particularly high corresponding to the regions which morphologically contained a vital growth plate (Figure 5). This was also true over the regenerated parts of the growth plates. In the most superficial zone, corresponding to that part of the transplanted cartilage which was superficial to the base level of the

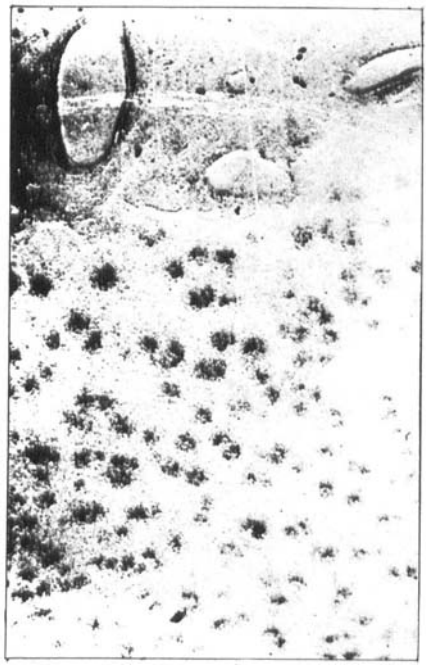

Figure 4 B. Detail from the area adjacent to the secondary centre of ossification shown in Figure 4 A (× 100). The labelling over the cells near the ossification centre is not significantly more pronounced than over the cartilage of the central areas. There is a marked concentration of granules over and around the cells, but there is also some labelling over the matrix.

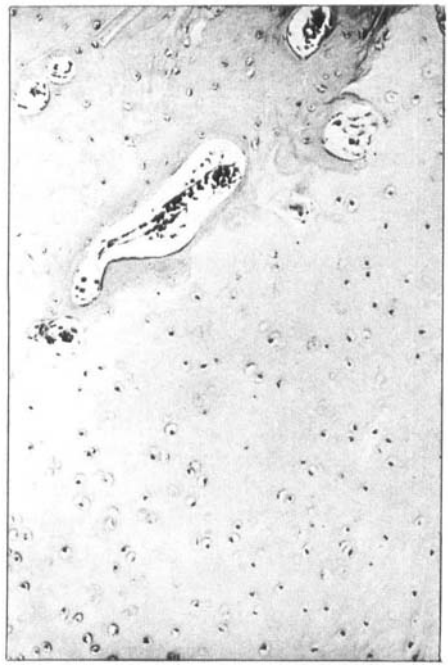

Figure 4 C. Morphological detail from the same area as Figure 4 B. (Haemalun-azophloxine-saffron, × 100). The cells near the ossification centre are not significantly swollen compared to the other cartilage cells.

surrounding joint cartilage, concentrations of granules generally appeared over and around the cells in the autoradiographs at all periods of observation, even at the longest ones (Figures 6 and 7). As to the quantity of granules within this part of the cartilage no obvious changes were seen to take place during the study performed.

Within the unlabelled regions heavily labelled islets were seen in some transplants. These obviously represented the metaplastically formed cartilage found in the morphological study.

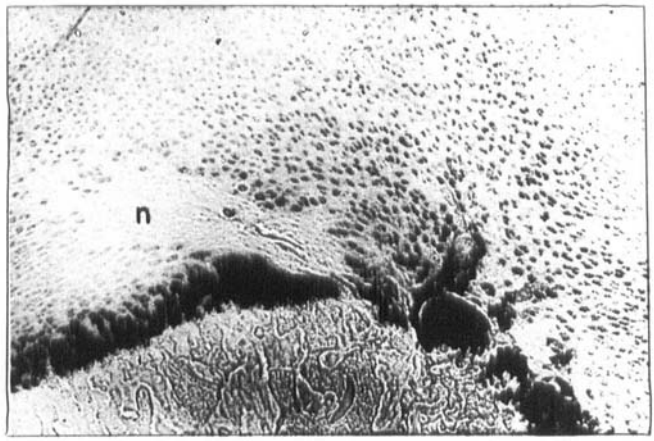

Figure 5. Autoradiograph of a section from a transplant to a load-bearing defect. Observation period: 6 weeks (× 25). The major part of the cartilage is vital. There is a lack of labelling only in a small region (n) in the basal area. The labelling is particularly high over the cells of the growth plate.

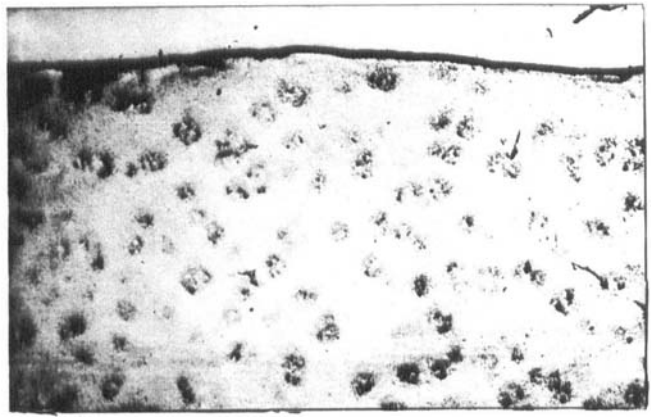

Figure 6. Autoradiograph of a section from the superficial part of a transplant to a load-bearing defect. Observation period: 6 months (× 100). The picture demonstrates a marked labelling over the cartilage, in particular over and around the cells.

Osteochondral transplants outside the joint surface

Within these transplants, unlabelled regions were found at about the same location and of the same size as in the transplants to the joint surface.

These areas corresponded well to the areas which morphologically appeared to be necrotic. Also in these transplants islets of tissue con-

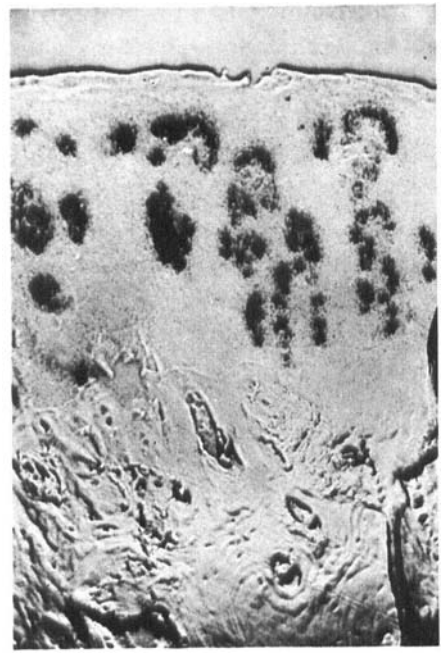

Figure 7. Autoradiograph of a section from a transplant to a load-bearing defect. Observation period: 14 months (× 100). There is heavy labelling over the cartilage, in particular over and around the cells.

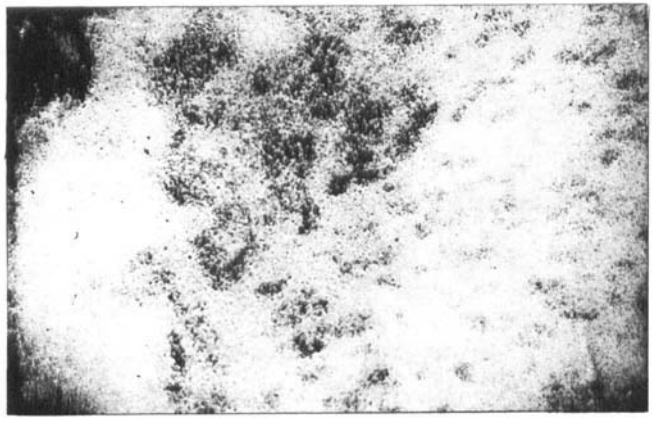

Figure 8. Autoradiograph of a section from a transplant to a defect outside the joint surface. Observation period: 12 weeks (× 100). The labelling over and around the cells is clearly demonstrated.

taining a heavy concentration of labelled sulphur were occasionally seen within such regions. The relation between the concentration of labelled sulphur within the morphologically vital superficial parts and that of the adjacent articular cartilage was definitely lower than in

the transplants to the joint surface. However, the cells of these parts of the transplants also undoubtedly concentrated labelled sulphur (Figure 8).

Osteochondral transplants with devitalized cartilage

There was no significant concentration of granules either over the chondrocytes or over the matrix of the cartilage which was of apophyseal origin. Corresponding to the ingrowing granulation tissue and to cartilage formed by metaplasia, however, there was a heavy concentration of granules, in particular over and around the most hypertrophic cartilage cells (Figure 9).

In all the autoradiographs some granules corresponding to the bone tissue were found, especially over the superficial parts of the subchondral trabeculae, and also over the interspaces. This labelling was, however, far less than that over the vital cartilage. In the control autoradiographs from animals that had not been injected with ^{35}S, no significant amounts of granules could be observed. The background activity was low, both in these controls and in the autoradiographs from animals given the isotope.

DISCUSSION

Autoradiography using ^{35}S has been applied by several workers as a functional test of the vitality of cartilage cells (Wyburn & Bacsich 1955, Curran & Gibson 1956, Craigmyle 1958, Gibson et al. 1958, Schatten et al. 1958, De Palma et al. 1963 and Hjertquist & Lemperg 1969) since Boström & Månsson (1952, 1953) found that the capability of cartilage to take up labelled sulphur and to incorporate it into chondroitin sulphate is dependent on an active enzymatic function of living chondrocytes and since Dziewiatkowski (1951 a) had visualized such uptake by autoradiographic technique.

In the present study the cartilage devitalized prior to implantation did not contain any significant amounts of ^{35}S. Furthermore, the labelling was mainly intra- and pericellular, whenever it occurred in joint cartilage, non-transplanted or transplanted apophyseal cartilage or metaplastically formed cartilage. These findings should indicate that the uptake of ^{35}S was due to functional activity of the cells. False negative labelling of the transplants was eliminated by discarding all sections where the adjacent joint cartilage was negative. False positive autoradiographs are unlikely for reasons mentioned above and because all the controls were negative. It has previously been shown that the

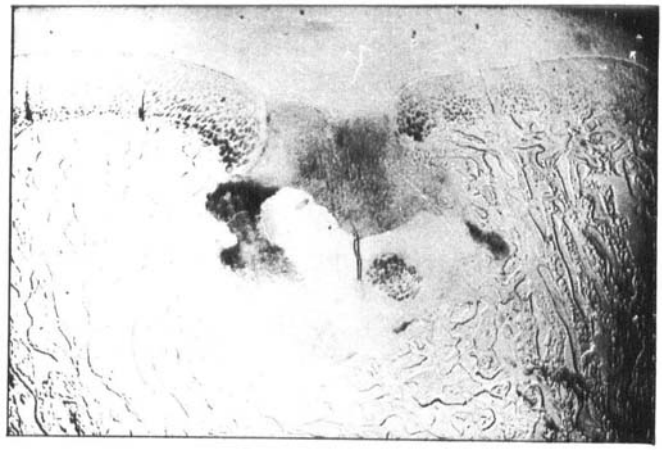

Figure 9 A. Autoradiograph of a section from a control defect filled with osseous transplant and devitalized cartilage. Observation period: 6 weeks (× 10).

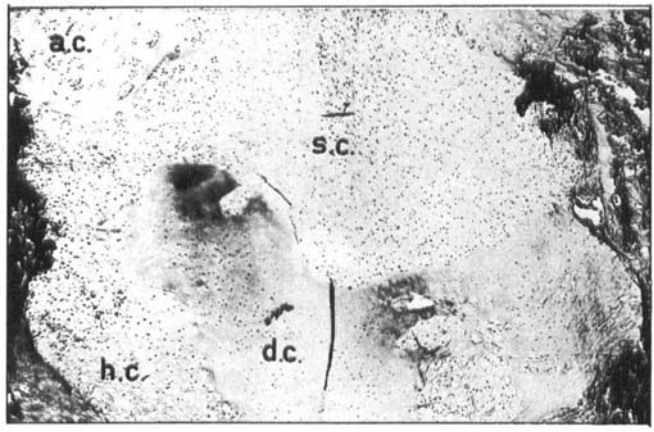

Figure 9 B. Morphological detail from the middle part of the defect shown in Figure 9 A (× 25).

The area of confluent labelling over the superficial part of the defect (Figure 9 A) reflects the radioactivity within the highly cellular and small-celled metaplastically formed cartilage (s. c.) shown in Figure 9 B. The areas of unevenly distributed labelling to the left of and within the pale unlabelled area in the deeper region represent the metaplastically formed cartilage containing hypertrophic cells (h. c.) shown in Figure 9 B. The pale unlabelled area of the autoradiograph corresponds to the remnants of devitalized cartilage (d. c.) in Figure 9 B. (a. c. = articular cartilage).

transfer of radioactively labelled sulphur compounds from the cells to the matrix starts even earlier than 48 hours after injection of the isotope

(Belanger 1954, Pelc & Glücksmann 1955). Hence, the present finding of considerable amounts of labelled sulphur in the matrix between the cells is most likely related to the length of the incubation period. Since most of the sulphur incorporated into cartilage is present in chondroitin sulphate (Dziewiatkowski 1951 b, Boström 1952) and the inorganic bound sulphur is removed by fixation in formalin (Campo & Dziewiatkowski 1961, Dziewiatkowski 1962) it seems reasonable to assume that most of the labelling in the cartilage in the present study is due to labelled sulphur incorporated into chondroitin sulphate.

The distribution of the labelling in the non-transplanted apophyseal cartilage was found to be similar to that registered in pressure epiphyses (Dziewiatkowski 1951 a), the labelling being most concentrated at or near the junction of the cartilage with the metaphysis and in the area surrounding the secondary centre of ossification. An increased concentration of labelled sulphur within the latter region was, however, no longer found when the initial stage of the secondary ossification had passed and hypertrophic cells were no longer seen. Thus a great part of the cartilage was ossified without the presence of increased cencentrations of labelled sulphuric compounds. This may suggest that the mechanism of preparation of the cartilaginous matrix for ossification had been altered from the early stages, since chondroitin sulphate is assumed to play a role in the determination of the calcifiability of the matrix (Herring 1972).

The investigation of the transplanted apophyseal cartilage confirms the findings of the morphological part of the study that apophyseal cartilage, except for some basal parts adjacent to the metaphyseal osseous part of the transplants, survives transplantation to osteochondral defects of joints, and under the given circumstances, also to the defects outside the cartilaginous joint surface. It further provides evidence that the cartilage cells do not only survive but also preserve their capacity for producing sulphuric compounds which are secreted into the matrix. Considering that these compounds most likely are mucopolysaccharides, mainly chondroitin sulphate, the importance of this observation is obvious if transplantation of apophyseal cartilage should be applied in restoring joint defects. The findings also showed that in particular the cartilage cells of the surviving parts of the growth plates, and growth plates which regenerated later on, produced heavy amounts of sulphuric compounds in a similar manner to the cartilage cells of the growth plates prior to transplantation. Finally, cartilage formed metaplastically within necrotic cartilage was found able to produce similar compounds.

SUMMARY

^{35}S was administered to 20 puppies which had been exposed to transplantation of osteochondral apophyseal transplants from the iliac crest to defects of the femoral condyles. Some transplants were implanted into defects within the joint surfaces whereas others were implanted outside the joint surfaces. An autoradiographic study was performed to assess the functional vitality of the transplanted cartilage at varying intervals up to 14 months. This study further necessitated an investigation of the normal pattern of incorporation of ^{35}S in the iliac crest.

The studies revealed that the incorporation of ^{35}S within the apophysis of the iliac crest was similar to that seen within pressure epiphyses, being heaviest in the proliferating and hypertrophying cells in the growth plate and around the secondary centre of ossification. The increased turnover of sulphur around the secondary centre of ossification declined, however, when the initial stage of the ossification was passed and when hypertrophy of cartilage cells was no longer seen. Ossification then took place without intensified production of organic sulphur-containing compounds in this region.

The study further showed that apophyseal cartilage was still able to incorporate ^{35}S following transplantation to the mentioned defects, except in some basal central areas adjacent to the metaphyseal bone. These findings suggested that the cartilage not only survived, but also preserved its capacity for synthetizing sulphur-containing compounds, probably chondroitin sulphate.

ACKNOWLEDGEMENTS

The author appreciates the technical help of Associate Professor Arne Attramadal, M.D. and Mrs. Marit Johanson, Institute of Pathology, Rikshospitalet, Oslo. Financial support was received from The Norwegian Research Council for Science and the Humanities.

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Key words: autogenous transplantation; autologous transplantation; epiphysis; articular cartilage; autoradiography

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