J. Anat. (1971), **109**, 2, pp. 317–327
With 14 *figures*
Printed in Great Britain

Repair of the joint surface from subarticular tissue in the rabbit knee

G. MEACHIM AND C. ROBERTS
Department of Pathology, University of Liverpool

(*Accepted* 19 *April* 1971)

The erosion of articular cartilage which occurs in osteoarthritis can lead to exposure of bone at the joint surface. Occasionally an area in which cartilage has been lost in this manner can subsequently acquire a new covering layer of non-osseous tissue, and within the new tissue a variable amount of cartilaginous metaplasia is sometimes apparent. In a previous study of human osteoarthritic femoral heads circumstantial evidence was presented that there are two potential sources for this repair process (Meachim & Osborne, 1970): mesenchymal tissue of synovial origin can spread on to the periphery of the joint surface, and mesenchymal tissue of subarticular origin can gain access to the more central areas of the surface through abnormal gaps in osseous continuity which are found in the subarticular bone plate. The present study is an investigation of repair by subarticular tissue in an experimental model in which the cartilage loss and bone plate defects of human osteoarthritis were artificially simulated by a surgical procedure carried out on one surface of the rabbit patello-femoral articulation. From the design of the experiment it was hoped to exclude repair from synovial tissue, and there was no evidence of repair from this source or from the rim of old cartilage which was retained around the margin of the operation site.

MATERIAL AND METHODS

The findings in 21 male rabbits (18 New Zealand White; three other breeds) are presented. Eleven of these animals were aged between 6 and 11 months at the time of operation; the other 10 were aged 1–3 years. A further three rabbits which were also subjected to surgery have been excluded from the results because of post-operative patellar displacement (two animals) or post-operative joint infection (one animal).

Surgical procedure

After shaving and cleaning the overlying skin, the lateral side of the patello-femoral articulation of the right knee joint was opened under ether anaesthesia. The full thickness of uncalcified articular cartilage was excised by scalpel from the patellar groove of the femur, except for a narrow rim of cartilage which was retained at the proximal, medial and lateral margins of the groove. The total surface area from which cartilage was removed averaged approximately 50 mm^2. No cartilage was taken from the femoral condyles or intercondylar notch. Using a metal awl rotated manually under pressure, holes, each 2 mm in diameter, were made through the subarticular

bone plate of the area from which the cartilage had been excised. Care was taken to space the holes apart from one another and to distribute them as evenly as possible over the area of cartilage removal; on the average five holes were made, the number being varied according to the size of the patellar groove in the animal concerned. The drill holes occupied approximately one-third of the total surface area from which the cartilage had been removed. Adequate depth of a hole was indicated by a sudden decrease in resistance during drilling, and by observing blood oozing into the defect after removal of the drill. The holes were then cleaned out with the point of a scalpel. The joint was closed using separate rows of sutures for the capsule and for the overlying skin. The sutured skin was covered by an Octaflex aerosol film. Following the operation the rabbits were housed in runs specially constructed to give the animal unrestricted movement over a floor area 150 × 60 cm.

Histological methods

The animals were killed at intervals of up to 120 weeks after the operation. After the right knee joint had been opened and inspected to confirm that there was no evidence of infection or of patellar displacement, the distal end of the femur was removed and fixed in buffered formol saline. After decalcification in ethylene diamine tetra-acetic acid (E.D.T.A.) the specimen was divided transversely into a consecutive series of blocks, each approximately 3·5 mm thick, cut at right angles to the articular face of the patellar groove. Four or five blocks were thus obtained, according to the length of the groove. The blocks were then processed and embedded in paraffin. Sections were cut from three different levels of each block, in order to obtain histological material representative of the whole of the patellar groove of the distal right femur. The sections were stained with Ehrlich's haematoxylin and eosin, and with 1 % toluidine blue diluted 1 in 10 in distilled water, followed by rinsing in water, blotting dry, and direct transfer to xylol.

On microscopy several different types of tissue were observed at the femoral articular surface. For each level of each block the findings were recorded separately for each microscopic field across the whole of the surface from which the articular cartilage had originally been removed; each field was aligned so as to include a surface segment of a constant length of 1·2 mm before magnification. The proportion of the total surface area of cartilage removal which had subsequently become covered by a layer of non-osseous repair tissue was then calculated. A variable amount of the surface repair tissue was cartilaginous, and the area covered by reparative cartilage was also estimated using the same method. When required, comparative data were obtained from the left knee of the experimental animals and from the right and left knees of normal rabbits.

RESULTS

In a joint from the immediate post-operative period the drill holes were represented histologically by gaps in the subchondral bone plate extending into the underlying cancellous bone (Fig. 1). The bony defects were empty apart from a small amount of blood clot and tissue debris in their bases. Examination of the joint surface adjacent to the drill holes at this and at later stages after the operation confirmed that the full thickness of the uncalcified articular cartilage had been removed, leaving compact

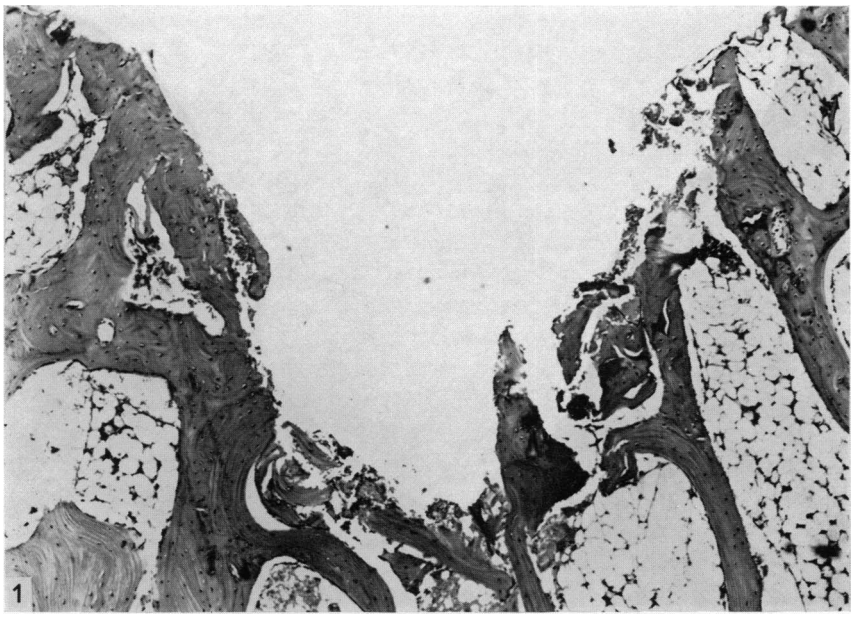
bone and foci of calcified zone cartilage exposed at the articular surface where this had not been drilled.

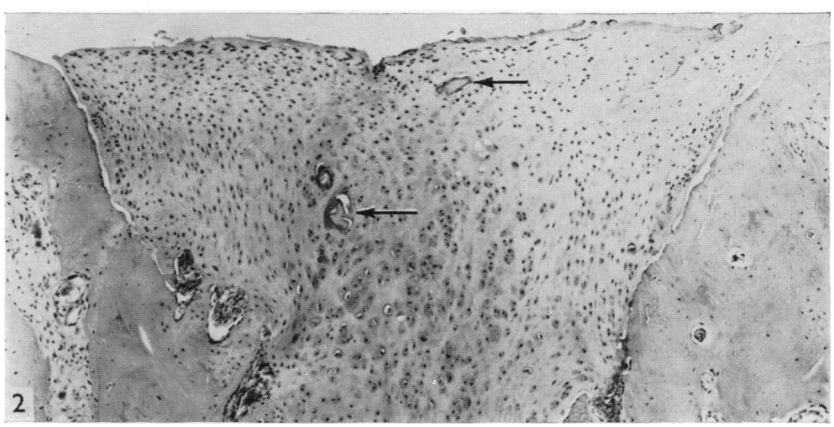
Two weeks after the operation the defects created by the drill holes were partially filled by richly vascular, loose-textured repair tissue and by more fibrous tissue. Formation of new bone, or of new bone with cartilage, was taking place at the base of the defects.

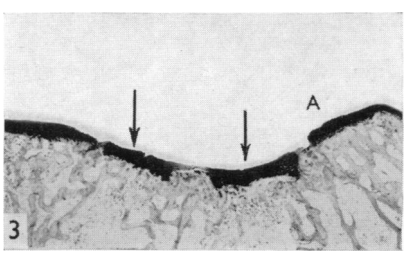
After nine or more weeks the drill holes were in nearly all instances completely occupied by new tissue which extended up to the level of the articular surface (Fig. 2); occasionally this new tissue had entrapped necrotic loose fragments of old cartilage and bone which had not been removed from the joint after being dislodged during the surgical procedure (Fig. 2). The gaps in bony continuity made by the drill holes were thus filled by fibrous tissue or often by cartilage and fibrous tissue (Fig. 2), and this new tissue presented an articular face at the joint surface. In parallel with this process there was evidence of continued osteogenesis or osteochondrogenesis at the bases and margins of the defects, and the plugs of non-osseous repair tissue thus became smaller as they were in turn progressively replaced by bone. After 18 or more weeks many of the non-osseous plugs had undergone complete or almost complete bony replacement at their articular surface (Fig. 3), with remodelling of the subarticular bone plate and restoration of its bony continuity at the site of the drill holes. After 60 weeks a persistent plug of cartilage or fibrous tissue within the bone was seen only occasionally. In some instances the new bone plate was reconstituted at a position slightly below its original level, giving a 'countersunk' appearance at one or both of its junctions with the old plate (Fig. 3). Exceptionally new non-osseous tissue did not completely fill in the defect in the manner which has been described, and instead the healed site of the drill hole showed an empty bowl-shaped depression surrounded by bone with a thin lining of fibrous tissue.

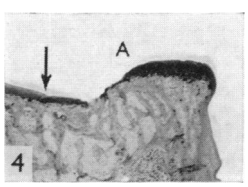
The surface region of the non-osseous repair tissue did not undergo bony replacement, but persisted as an articular layer between the reconstituted bone plate and the joint cavity (Fig. 4). Moreover, this non-osseous repair tissue often spread over the adjacent non-drilled joint surface from which the original articular cartilage had been surgically excised (Fig. 5); the growing edge tended to show an irregular outline in transverse section. In many animals this tongue of new tissue (Fig. 5) fused in places with the narrow rim of old cartilage which had been retained around the margins of the operation site (Fig. 6). The total amount of surface repair was measured separately for each animal, and the results are shown in Fig. 7; it will be seen that the proportion of the surface covered by non-osseous tissue tended to increase with time, but that the results varied from animal to animal and that complete re-covering was exceptional. This variability was due partly to a greater potential for repair in the younger rabbits (Fig. 7). The results represent surface repair tissue at the site of the drill holes together with any tissue which had spread over the adjacent exposed joint surface; since the drill holes originally occupied approximately one-third of the total surface area from which cartilage had been removed, any covering in excess of that amount can be attributed to spread from the site of the holes (Fig. 7). At nine weeks after the operation most of the repair tissue represented the articular face of non-osseous plugs in the bone plate (Fig. 2), but by 18 weeks most or all of it had acquired a well-formed support of underlying bone (Fig. 4).

No evidence was seen of reparative covering originating from the old cartilage which had been retained at the margins of the operation site; this cartilage sometimes developed multicellular rounded clusters. There was also no evidence of spread of synovial tissue across the rim of old cartilage on to the surface of exposed bone. In









the present experiments the surface repair was effected by tissue which had formed in the drill holes; although tiny foci of new fibrous tissue and of new cartilage were sometimes seen where cartilage removal had exposed intertrabecular spaces on a non-drilled area of the surface (Fig. 5), these foci and any foci of calcified zone cartilage which had been left on the surface made no significant contribution to the reparative process.

The histological pattern of the surface repair tissue varied from area to area, and the relative proportions of the different histological types varied from animal to animal. The tissue was classified as 'cartilaginous' and 'non-cartilaginous' on the basis of the morphology and arrangement of its cells and on the basis of the appearance of its intercellular matrix in sections stained with toluidine blue. Since the development of a cartilaginous texture amongst the repair tissue was represented by a gradual rather than an abrupt change in histological pattern, the distinction between 'cartilage' and 'non-cartilage' was to some extent subjective and arbitrary. Tissue was recorded as 'cartilaginous' if it contained chondrocyte-like cells and showed a positive staining reaction with toluidine blue in a major part of its matrix (Fig. 6); matrix staining was absent in the superficial layer in some areas included in this category (Fig. 8). The rest of the repair tissue was recorded as 'non-cartilaginous': some of this was fibrous; some was an 'intermediate type' between that of fibrous tissue and cartilage (Fig. 9), and some was 'chondroid', containing a predominance of rounded chondrocyte-like cells but showing no staining with toluidine blue throughout the major part of its matrix (Fig. 10). The articular aspect of the fibrous and 'intermediate type' tissue was often irregular in contour (Fig. 9). That of the cartilage and 'chondroid' usually appeared smooth when examined by light microscopy (Figs. 6, 8 and 10), although in a minority of animals it showed small foci of superficial fraying.

The amount of cartilaginous repair at the articular surface was estimated separately for each animal; the results are shown in Fig. 11. It will be seen that there was considerable variation from animal to animal even when the results were taken separately for the younger and older rabbits. There was no close relationship between the amount of new cartilage and the total amount of non-osseous surface repair (Figs. 7 and 11), or between the amount of new cartilage and the time after operation (Fig. 11).

The new cartilage was more often found as focal plaques than as a continuous sheet. In sections stained with toluidine blue its texture could nearly always be

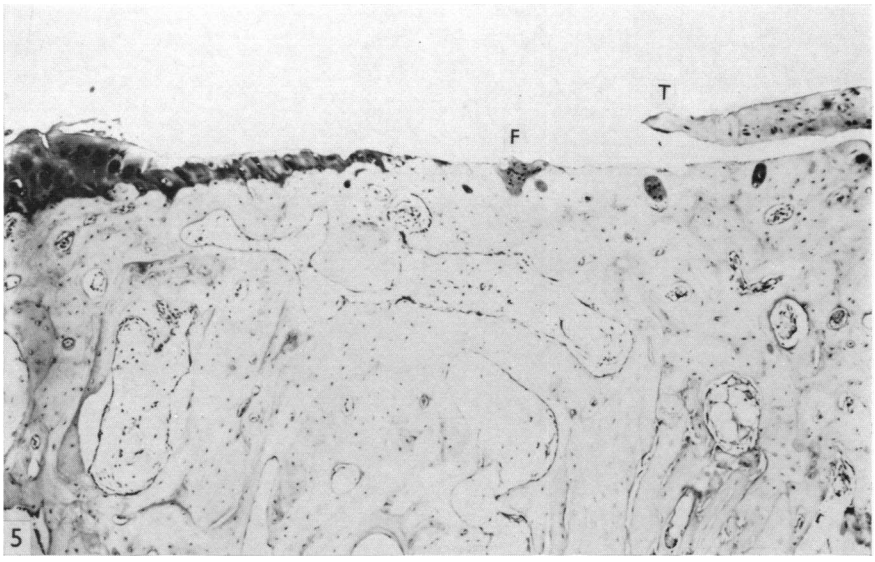
---

Fig. 1. One of the drill holes immediately following the surgical procedure, showing the defect made through the subchondral bone plate into cancellous bone. This and all subsequent photomicrographs are from sections cut transversely across the femoral surface of the rabbit patello-femoral articulation. Haematoxylin and eosin, × 60.

Fig. 2. A drill hole 9 weeks after the surgical procedure, now filled by non-osseous repair tissue which extends up to the level of the articular surface. Note the entrapped necrotic fragments of tissue dislodged at the time of the operation (arrows). Haematoxylin and eosin, × 60.

Fig. 3. The site of two drill holes 24 weeks after the surgical procedure (arrows). The non-osseous plugs (Fig. 2) have undergone bony replacement except at their articular surfaces. Note the 'countersunk' appearance at the junctions of the reconstituted bone plate with the original subchondral plate. Edge of old cartilage (A). Toluidine blue, × 5.

Fig. 4. Twenty-three weeks after the surgical procedure, showing an articular layer of non-osseous repair tissue (arrow) with underlying bone. Edge of cartilage (A). Toluidine blue, × 5.



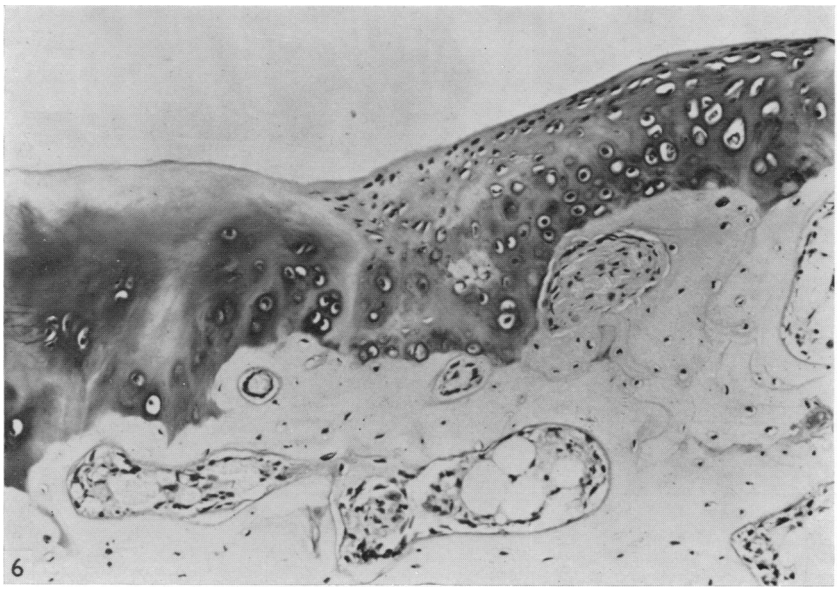
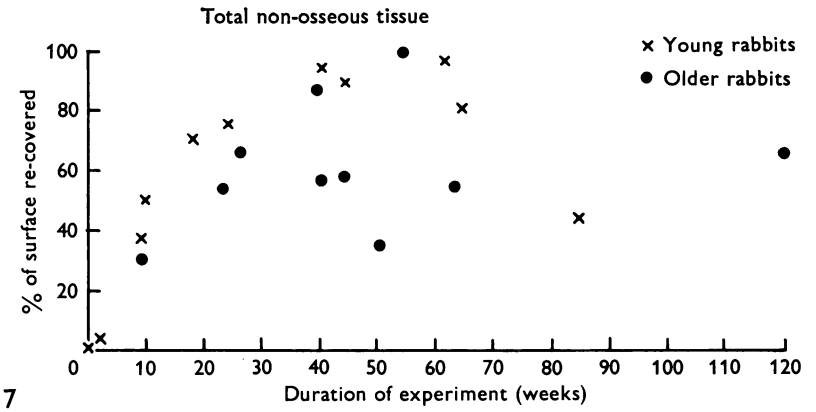
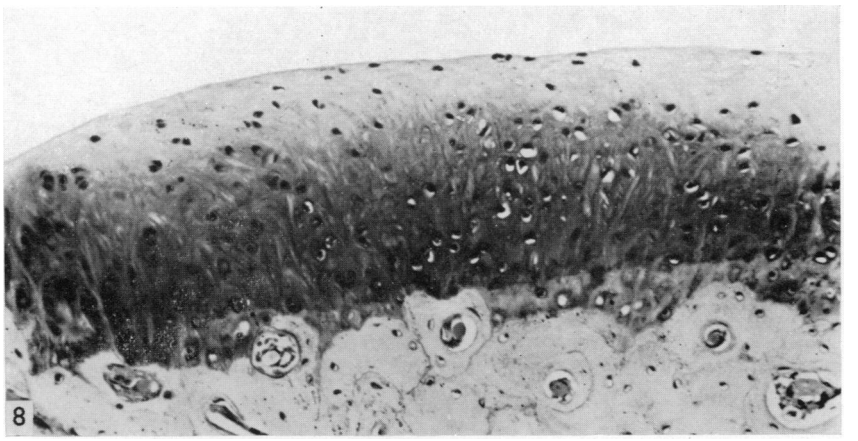
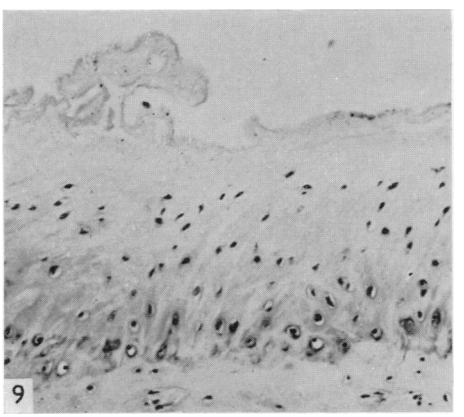


Fig. 5. Tongue of new tissue (T) growing over a non-drilled area of the joint surface from which the original cartilage had been excised. Note also the tiny focus of new tissue (F) which has formed where cartilage removal had exposed an intertrabecular space in the bone plate. The edge of the original cartilage, partially excised, is seen on the left of the field. Twenty-three weeks. Toluidine blue, × 60.

Fig. 6. Non-osseous articular repair tissue (right) most of which is cartilaginous, fused with the rim of old cartilage (left) retained at the margin of the operation site. In this field the new cartilage is virtually hyaline in texture (compare with Figs. 12 and 13). Forty weeks. Toluidine blue, × 150.







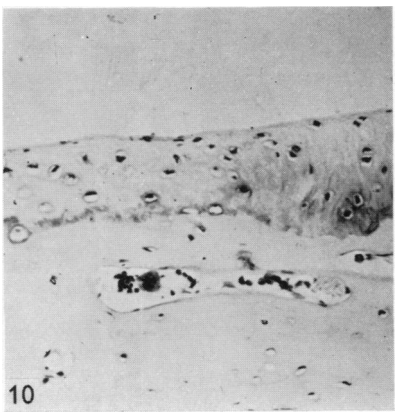
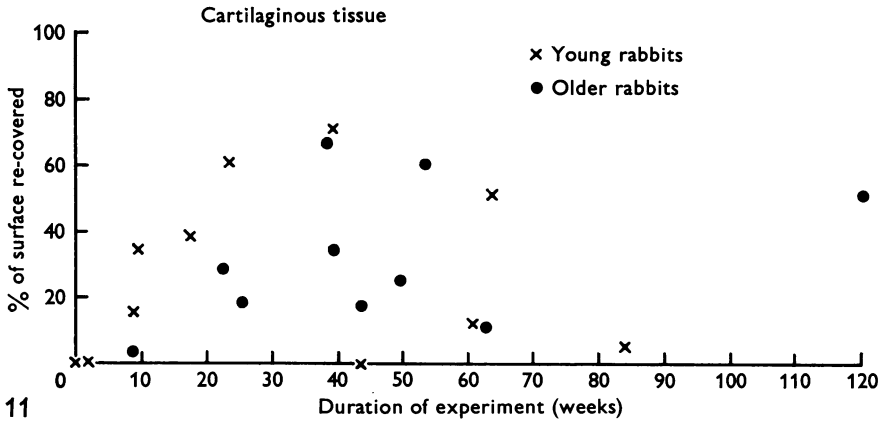


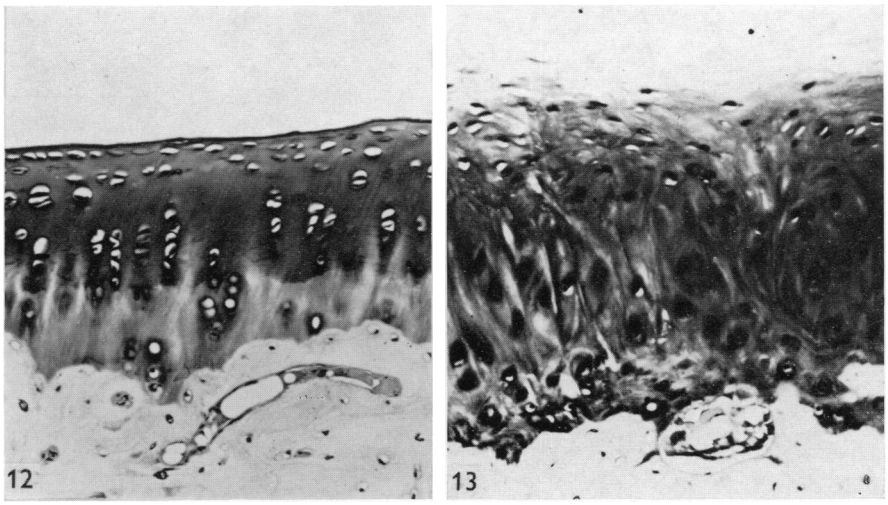
Fig. 7. Percentage of the total surface area of cartilage removal re-covered by non-osseous repair tissue in relation to the time after the surgical procedure. The results are shown separately for the young rabbits (age range 6–11 months at the start of the experiment) and the older rabbits (age range 1–3 years).

Fig. 8. Fibrocartilaginous repair tissue in which matrix staining is absent in the superficial layer. Note the sharp boundary with the discontinuous layer of calcified cartilage adjacent to bone. Forty weeks. Toluidine blue, × 150.

Fig. 9. Repair tissue of 'intermediate' type. Note the irregular contour at the articular surface, especially on the left of the field. Sixty-four weeks. Toluidine blue, × 150.

Fig. 10. Repair tissue mainly of 'chondroid' type. Compare with Fig. 8. Sixty-one weeks. Toluidine blue, × 150.





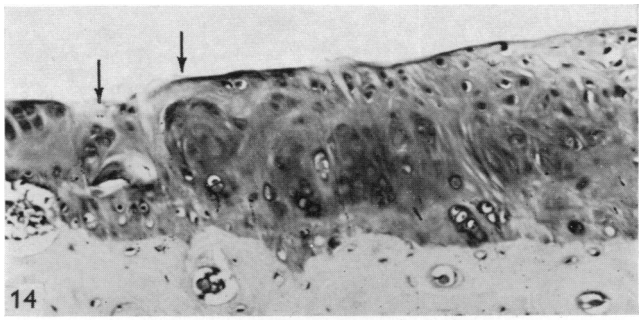


Fig. 11. Percentage of the total surface area of cartilage removal re-covered by repair tissue of cartilaginous texture, in relation to the time after the surgical procedure. The results are shown separately for the young rabbits (age range 6–11 months at the start of the experiment) and the older rabbits (age range 1–3 years). Compare with Fig. 7.

Fig. 12. Control section of mature hyaline articular cartilage. Note the sharply defined boundary between the calcified zone and the overlying uncalcified tissue. Femoral surface of a patello-femoral joint not subjected to surgery. Toluidine blue, × 150.

Fig. 13. Reparative fibrocartilage. Compare with Fig. 12. Twenty-four weeks. Toluidine blue, × 150.

Fig. 14. Reparative fibrocartilage showing in places an irregular arrangement of cells as small clusters or curved formations (arrows). Note the calcified zone, but compare with Fig. 12. Forty weeks. Toluidine blue, × 150.

distinguished from that of mature hyaline articular cartilage. Thus, in control sections from the patellar groove of the femur of adult rabbits all the uncalcified cartilage, except that immediately adjacent to the proximal margin of the groove, was hyaline in texture with an intercellular matrix of a homogeneous, 'ground glass' appearance (Fig. 12). In contrast, the matrix of the repair cartilage nearly always showed a 'streaky', fibrocartilaginous texture on staining with toluidine blue (Figs. 8 and 13), although exceptionally small foci with a hyaline pattern were seen (Fig. 6). In sections stained with Ehrlich's haematoxylin and eosin this difference in texture was often not apparent.

In some areas the cells were distributed regularly throughout the repair cartilage; in others they tended to be arranged in small clusters or curved formations (Fig. 14). In the repair cartilage and 'chondroid' the cells immediately below the surface often presented an elongated, flattened appearance in the plane of section (Fig. 6), as in normal joints. Control sections of hyaline articular cartilage showed a well-demarcated calcified zone in which the staining intensity with toluidine blue differed from that of the overlying uncalcified cartilage (Fig. 12); a smoothly linear 'tide mark' was seen at the interface between calcified and uncalcified tissue. In many areas of the repair cartilage a basal calcified zone was less easily made out than in the control sections (Fig. 13); where it was apparent, the calcified zone showed either an irregular or a smoothly linear boundary with the uncalcified tissue (Figs. 8 and 14).

DISCUSSION

Several observers have independently demonstrated that the cells of mature hyaline articular cartilage in man and in the rabbit do not usually effect repair of an area of cartilage loss at a joint surface (Collins, 1949; Landells, 1957; DePalma, McKeever & Subin, 1966; Campbell, 1969). The present observations are in keeping with this finding, since there was no evidence of any reparative covering originating from the rim of old cartilage retained at the margins of the operation site. The cells in the rim of cartilage sometimes formed multicellular rounded clusters: this phenomenon represents a 'reactive' response by chondrocytes adjacent to a cartilage incision (Carlson, 1957; Meachim, 1963), but the response does not normally progress to actual repair.

There are, however, also extrinsic sources for potential repair of an articular surface by a new layer of non-osseous tissue. Mesenchymal tissue of synovial origin can spread on to the peripheral part of the surface in joints which have been damaged by trauma (Landells, 1957) or by osteoarthritic changes (Meachim & Osborne, 1970). This phenomenon was not seen in the present study: possibly the rim of old cartilage intentionally preserved at the margins of the operation site can act as a barrier to encroachment by synovial tissue in joints where movement is unrestricted.

Another potential source of surface repair is from the mesenchymal tissue of the subarticular bone plate. This can occur in man following intra-articular trauma (Landells, 1957) and following drilling of the bone plate during surgical débridement of a joint (Insall, 1967). Similarly, there is circumstantial evidence from histological studies in man that tissue of subarticular origin can sometimes gain access to the surface of an osteoarthritic femoral head through abnormal gaps in osseous con-

tinuity which are found in the subarticular bone in this condition (Meachim & Osborne, 1970); a variable amount of cartilaginous metaplasia can occur in the non-osseous repair tissue. Meachim & Osborne (1970) have suggested that in favourable circumstances bone remodelling in the osteoarthritic femoral head can subsequently restore bony continuity across the gap, and thus provide a base of new bone for the surface repair tissue; it has also been suggested that the non-osseous repair tissue can spread over exposed bone surfaces adjacent to the defects in the subarticular plate. The present observations provide direct evidence in support of these concepts, since in the experimental situation studied in the rabbit it was possible to observe the reparative process at different stages and thus to demonstrate the sequence of its development. DePalma, McKeever & Subin (1966) have previously demonstrated repair of defects made by boring out a cylindrical plug of canine articular cartilage with underlying bone; in their experimental study the cartilage on the joint surface adjacent to the holes was not excised, and the possibility of spread of non-osseous repair tissue over an exposed bone surface was not investigated. Chesterman & Smith (1968) have observed fibrous tissue of subarticular origin at the base of full-thickness articular cartilage defects made surgically on the humeral head in rabbits; they noted no apparent change in the size of the defects, but did not study them for periods of longer than six weeks after the operation.

In human osteoarthritis erosion of bone following loss of articular cartilage can cause intertrabecular spaces in the osseous plate to become exposed at the articular surface. Small foci of loose-textured or fibrous tissue can develop at the surface in such areas (Meachim & Osborne, 1970). Similarly, in the present experiment small foci of new fibrous tissue and of new cartilage were sometimes seen where surgical excision of the cartilage had exposed intertrabecular spaces on a non-drilled area of the surface. Such foci made no significant contribution to the repair process in the animals studied, and surface repair was effected from the frank defects created in the osseous plate by the drill holes. This finding suggests that drilling deeply into the subarticular plate would increase the likelihood of subsequent repair in operations on a joint surface in man. The comments made by Insall (1967) on techniques employed in joint débridement are in line with this suggestion; it may also be relevant to the techniques used in the surgical treatment of osteochondritis dissecans.

A variety of histological types of repair tissue can occur at the articular surface in osteoarthritis of the human hip, and the various types are often intermingled in topographical distribution on the femoral head. A similar variety and intermingling of histological types has been produced experimentally in the present study, as in that reported by Campbell (1969). Although the 'intermediate' and 'chondroid' types could represent divergent pathways of tissue differentiation, it seems more likely that they represent progressive stages in the development of a cartilaginous texture.

SUMMARY AND CONCLUSIONS

Repair of the articular surface of a synovial joint by mesenchymal tissue of subarticular origin has been investigated experimentally in a series of 21 male rabbits. The full thickness of uncalcified articular cartilage was removed surgically from most of the femoral surface of the patello-femoral component of the knee joint, and

a number of holes were drilled into the subarticular bone plate of the same area. The changes at the operation site following this procedure were then studied at intervals of up to 120 weeks after the operation.

Non-osseous repair tissue grew into the drill holes which had been made in the bone plate, and extended up to the level of the articular surface. This repair tissue was progressively replaced by bone, with remodelling of the subarticular osseous plate and restoration of its bony continuity at the site of the holes. The surface region of the non-osseous repair tissue did not undergo bony replacement; it persisted as an articular layer between the reconstituted bone plate and the joint cavity, spread on to the adjacent non-drilled joint surface, and in places fused with the narrow rim of cartilage which had been intentionally retained around the margins of the operation site. This rim of the original cartilage did not contribute to the repair process. Quantitative studies showed that the area of the articular surface covered by repair tissue tended to increase with time, but there was variation from animal to animal and complete re-covering was exceptional. The surface repair tissue comprised variable proportions of fibrous tissue, 'intermediate type' tissue, chondroid, and cartilage. The cartilage was more often seen as focal plaques than as a continuous sheet. On toluidine blue staining it nearly always appeared to be fibro-cartilaginous rather than hyaline in texture.

We are grateful to Mr John Massey, Mr James Smith and Mr David Williams for technical assistance.

REFERENCES

CAMPBELL, C. J. (1969). The healing of cartilage defects. *Clin. Orthop.* **64**, 45–63.

CARLSON, H. (1957). Reactions of rabbit patellary cartilage following operative defects. *Acta orthop. scand.* Suppl. 28.

CHESTERMAN, P. J. & SMITH, A. U. (1968). Homotransplantation of articular cartilage and isolated chondrocytes. *J. Bone Jt Surg.* **50B**, 184–197.

COLLINS, D. H. (1949). *The Pathology of Articular and Spinal Diseases*, pp. 59–61 and 74–115. London: Arnold.

DEPALMA, A. F., MCKEEVER, C. D. & SUBIN, D. K. (1966). Process of repair of articular cartilage demonstrated by histology and autoradiography with tritiated thymidine. *Clin. Orthop.* **48**, 229–242.

INSALL, J. N. (1967). Intra-articular surgery for degenerative arthritis of the knee. A report of the work of the late K. H. Pridie. *J. Bone Jt Surg.* **49B**, 211–228.

LANDELLS, J. W. (1957). The reactions of injured human articular cartilage. *J. Bone Jt Surg.* **39B**, 548–562.

MEACHIM, G. (1963). The effect of scarification on articular cartilage in the rabbit. *J. Bone Jt Surg.* **45B**, 150–161.

MEACHIM, G. & OSBORNE, G. V. (1970). Repair at the femoral articular surface in osteoarthritis of the hip. *J. Path. Bact.* **102**, 1–8.