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REACTIONS OF RABBIT PATELLARY CARTILAGE FOLLOWING OPERATIVE DEFECTS

A MORPHOLOGICAL AND AUTORADIOGRAPHIC STUDY

BY

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PREFACE

My former chief Professor ANDERS WESTERBORN, M. D., Director of the Department of Surgery, Section II, Sahlgrenska Sjukhuset, Gothenburg, and Professor BO-ERIK INGELMARK, M. D., Director of the Institute of Anatomy, University of Gothenburg, gave me the impulses to commence the investigation here presented. I am much indebted to Professor WESTERBORN for his encouragement and kindness during the initial stages of the investigation.

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CHAPTER I

INTRODUCTION

The literature on articular cartilage reactions to operatively created defects is rather confusing. Inadequate numbers of experimental animals have been used for many investigations; and the observation periods have often been too short. Moreover, in most cases only routine histological techniques have been used and the staining procedures have been too few and unselective.

The regenerative capability of articular cartilage is such a wide problem that only a part of it has been studied in the present investigation. This study is thus confined to the intrinsic ability of the patellary cartilage to react following operative interventions.

The aim of the present investigation was that it should answer the following questions: In what manner, how regularly and after how long does the rabbit patellary cartilage react to operative lesions? And is it possible by exogenous factors somehow to modify whatever reaction there might be?

These problems were approached along two general lines. In a first series of experiments rabbit knee joints were examined using regular histological procedures supplemented by some selective staining techniques. This phase of the investigations is discussed in Part I of this work.

In a second series of experiments the problem of articular cartilage reaction to surgically created defects was penetrated more deeply with the aid of a quite different technique, namely autoradiography with radiosulphate. This technique enables the sulphate metabolism of the articular cartilage to be studied.

S^{35} has been found extremely well suited for studying the

sulphate metabolism of cartilage. This isotope was therefore used for recording autoradiographs from operated and unoperated articular cartilage since there have been some gaps in our knowledge regarding the sulphate metabolism of such cartilage. The scope of the investigation was consequently broadened to cover the following questions: How is S^{35} distributed in articular cartilage at different times after the injection? How is S^{35} distributed in the various articular cartilage strata? Does the S^{35} uptake of operated articular cartilage differ from that of unoperated? Are the cell density in and S^{35} uptake of articular cartilage correlated? Is there any relationship between metachromasia and S^{35} uptake of articular cartilage? Part II deals with the results of experiments designed to answer these questions.

PART I

MORPHOLOGICAL STUDIES

CHAPTER II

SURVEY OF THE LITERATURE

Morphology and Physiology of Articular Cartilage

Articular cartilage is a quite specialized, dense, tough, compressible, mesenchymal tissue. It is composed of cells and intercellular matrix. The cells or chondrocytes are situated in lacunae or spaces in the matrix, and often an aggregate of two or more cells form what is known as a chondron. In articular cartilage four morphological strata can be distinguished, namely (i) the calcified cartilage stratum facing the bone, (ii) the radiate stratum, (iii) the intermediate stratum and (iv) the superficial or tangential stratum. This subdivision of the cartilage is based on the arrangement of its chondrons and collagen fibres (Fig. 1).

The chondrocytes have various shapes — superficially they are flattened, deeper down they are ellipsoid or polygonal. Their nucleus is of spheroid form and contains one or more nucleoli. The articular cartilage lacks perichondrium but, according to FISHER (1922—23) and other workers, the synovial membrane in a joint is tucked in and attached to a narrow border of each cartilage and so may be said to form a sort of marginal perichondrium.

It has long been held that fat exists in old chondrocytes (WEICHSELBAUM, 1872; RÖSSLE, 1923) and during inflammatory conditions (SCHMAUS, 1912). More recent investigations have revealed, however, that fat droplets occasionally can be observed in developing cartilage (CLARK & CLARK, 1942; SHEEHAN, 1948). Moreover, in MONTAGNA's (1949) opinion, the fact that fat has been observed in cartilage from newborns and in immature chondrocytes tends to disprove the hypothesis that the presence

of intrachondrocytic fat is itself a sign of degeneration. He postulated that such fat might have been built up from the carbohydrates in these cells, a theory partly based on SCHAFFER's (1930) finding that in embryonic chondrocytes storage of glycogen precedes that of fat. So far as I am aware, modern literature has nothing to say on the relative amounts of fat in degenerated and regenerating cartilage.

Normal chondrocytes have a high glycogen content. Indeed, in adult mammals the glycogen content of cartilage is surpassed only by the liver and muscles (WELLS, 1920). At all stages of postnatal development, glycogen in the form of minute perinuclear granules can be demonstrated in the chondrocytes but, notably, the number of these granules may vary between very wide limits (MONTAGNA, 1949). According to MANCINI (1948), after freeze-drying the glycogen will be found uniformly distributed throughout the cytoplasm while the use of chemical fixation agents almost invariably causes the glycogen to occur as irregular clots or granules and occasionally may result in extracellular diffusion of glycogen.

The matrix consists of an amorphous intercellular material, the ground substance, through which pass numerous collagen fibres, the formed intercellular substance. Having great tensile strength, these collagen fibres are probably formed by the chondrocytes, but whether the formation takes place intracellularly or extracellularly has been a moot point in recent years. Yet one thing seems certain, the ultimate phase of the formative process occurs at the cell surface (KULONEN, 1954).

Numerous investigators have studied the arrangement of the collagen fibres in the articular cartilage. Using a variety of methods, HAMMAR (1892) and HULTKRANTZ (1897) examined the fibre structure of and crevice formation in cartilage, finding that cracks in the cartilage surface tended to indicate the internal arrangement of the fibres. To BENNINGHOFF (1925), however, goes the credit for establishing the facts that the collagen fibres are arranged spatially as archlike hoops and that in the cartilage margins they are connected with the periosteal tangential fibres. The collagen fibres in cartilage are

attached to the floor of the calcified stratum but lack direct connections with the osseous collagen fibre system.

According to BANFIELD (1954), NEUBERGER & SLACK (1953) have demonstrated that collagen in rat tissue — the protein of which, as the name implies, collagen fibres are composed — »is relatively inert metabolically». They drew this conclusion from the results of experiments with labelled glycine which indicated that once collagen has been laid down its turnover is very slow.

Ordinary haematoxylin-eosin staining of articular cartilage is incapable of revealing the collagen fibres because these are masked by the mucopolysaccharide of which the ground substance is made up.

The ground substance of the intercellular matrix consists, as just mentioned, of a mucopolysaccharide. It is chondroitin sulphuric acid and occurs in the cartilage as a very viscid gel (HAM, 1953).

MEYER & coworkers (1937) and SYLVÉN (1947) maintained that, generally speaking, mucopolysaccharides are bound to the surface of collagen fibres. In discussing chondroitin sulphates, MEYER (1954) stated ». . . they are probably in intimate contact with the fibrous elements and actually may form part of the fibrous structures». The mucopolysaccharides among other things impart toughness to the cartilage (HIRSCH, 1944; WISLOCKI, BUNTING & DEMPSEY, 1947). In those parts of the articular cartilage that have to withstand considerable pressure the ratio of mucopolysaccharides to collagen is higher than in lightly stressed parts (MATTHEWS, 1953).

While the absolute amount of collagen probably rises with advancing age, the ratio of mucopolysaccharides to collagen per unit volume diminishes (SYLVÉN & MALMGREN, 1952). A number of investigators including MATTHEWS (1953) have observed that loss of mucopolysaccharides and emergence of collagen fibres — a process called fibrillation — are the first morphological changes exhibited by articular cartilage in osteoarthritis. According to the latter author, articular cartilage with such lesions has a subnormal ratio of chondroitin sulphate to collagen.

Metachromatic staining techniques have proved valuable adjuncts for demonstrating the presence of mucopolysaccharides in tissues.

After developing a method for determining the presence and distribution of sulphuric acid esters of high-molecular alcohols in tissues, LISON (1935) showed that certain dyes have a characteristic effect upon these esters and that tissues containing them are stained metachromatically. This and the fact that cartilage contains but a single sulphuric acid ester, i. e. chondroitin sulphuric acid, caused HIRSCH (1944) to adopt Lison's procedure in a study of chondroitin sulphuric acid in cartilage. By histological and clinical means he found that chondromalacia patellae was accompanied by depletion of mucopolysaccharides. Furthermore, he found that in this disorder the reduction of metachromasia parallelled the degenerative changes of the patellary cartilage.

Initially, the metachromasia was attributed to the SO_4-radical in the mucopolysaccharide. However, not long after, WISLOCKI, BUNTING & DEMPSEY (1947) demonstrated that the hyaluronic acid present in the tissues also possessed metachromatic staining properties, though in the case of the sulphated polysaccharide the colouration was brighter and more constant. SYLVÉN (1947) reported that the intensity of metacromasia produced by Lison's technique for toluidine blue staining can be taken as a measure of the chondroitin sulphate content of articular cartilage. This is not incompatible with WISLOCKI & COWORKERS' finding that metachromasia is unspecific for sulphated polysaccharides: chondroitin sulphate is the only component of cartilage that stains metachromatically. Nevertheless, PERSSON (1953) has shown that metachromasia in general and that due to hyaluronic acid in particular are highly susceptible to the quality of the dye used. Consequently, in carrying out comparative analyses of metachromatic intensity, it is highly important to use identical preparation procedures and the same stock solution in one series of observations. The results of SYLVÉN, HIRSCH, etc. can thus be expressed as follows — diminished or absent metachromasia of articular cartilage is evidence

of degenerative changes resulting from reduced chondroitin sulphuric acid content.

It appears from recent findings that chondroitin sulphate occupies a central position in the biology of articular cartilage. This was pointed out especially by SYLVÉN (1947) who attributes to this substance major roles in the metabolism and function of articular cartilage.

Although no conclusive proof has been furnished, a number of authors hold the view that chondroitin sulphate is produced by the chondrocytes. This is borne out by the results of recent investigations using radioactive sulphur. The outcome of such isotope studies and also the chondroitinsulphate metabolism in cartilage will be discussed more fully in Part II.

Nutrition of Articular Cartilage

In discussing the ways by which nutrients may be transported to the articular cartilage, EKHOLM (1951) distinguished two main routes, namely via (1) the blood vessels and (2) the synovial fluid, singly or in combination.

Nutrition via blood vessels. The blood vessels in question are those situated near the edge of the articular cartilage, that is in the marginal portions invested by the synovial membrane. It is identical with the ring of vessels HUNTER (1743) called the »circulus articuli vasculosus». In addition there exist fairly intimate contacts between the bone marrow's blood supply and the calcified stratum of articular cartilage (HOLMDAHL & INGELMARK, 1948). Also INGELMARK & SÄÄF (1948) emphasized that the subchondral vessels have an important function in the metabolism of articular cartilage. Apart from the marginal vessels the articular cartilage has no blood or lymphatic vessels of its own.

Nutrition via synovial fluid. Long ago, VIRCHOW (1863) and SCHMIEDEN (1900) maintained that loose intraarticular bodies received substances essential to their metabolism from the synovial fluid and so could remain viable and perhaps even grow. KLING (1931) pointed out that the presence of loose

bodies in the joint stimulated the joint capsule to enhanced transudation of proteins and carbohydrates from the vessels of the synovial membrane, providing the loose bodies with a more nourishing substrate than otherwise would have been the case. After introducing into the joint cavity of experimental animals loose cartilage-and-bone bodies taken from the cartilage, BENNETT, BAUER & MADDOCK (1932) found that chondrocytes still remained viable 28 weeks later, at which time the osseous tissue had become necrotic. Notably, however, their publication makes no reference to the significance of synovial fluid for the nutrition of articular cartilage.

Nutrition via blood vessels as well as synovial fluid. FISHER (1939) reported that the central superficial parts of the articular cartilage receive nutrients chiefly from the synovial fluid, the peripheral portions from circulus articuli vasculosus and the deeper central parts from vessels in the subchondral bone. EKHOLM (1955) drew the following conclusions: the articular cartilage is nourished partly through the joint surface, that is from the synovial fluid, partly through the juxta-epiphysial surface. While neither the absolute nor the relative amounts of nutrients mediated by the two surfaces could be established, the evidence suggested that the basal route was the more important.

The concensus of today's opinion seems to be that the subchondral and marginal blood vessels dominate in the nutrition of respectively the basal and peripheral portions of the articular cartilage while its central and superficial parts are supplied mainly from the synovial fluid.

Diffusion of nutrient fluid through the articular cartilage. Lacking blood vessels and lymphatics, the articular cartilage was believed by the first protagonists of modern histology to possess a finely subdivided network of preformed canaliculi for conveying nutrient fluids to all parts of the tissue. HANSEN (1900) and SCHAFFER (1930) have demonstrated, however, that the idea that there exists a system of such canaliculi is based on misinterpretation of a variety of artefacts.

Some researchers, for example LUBOSCH (1910) and SACKS

(1949), have contended that nutrients are spread intrachondrally owing to percolation along the intercellular fibril structures. BAUER, ROPES & WAINE (1940) pointed out that the structure of the intercellular chondral matrix resembles that of foam rubber and that dyes are capable of spreading through it by diffusing along the fibrils.

SCHAFFER's (1930) opinion was that nutrition of the cartilage — a slow and centrally inadequate process — is accomplished by osmosis through the intercellular matrix.

Mechanical factors may have a marked effect on the nutrition of articular cartilage. HILDEBRAND (1921), MÜLLER (1929), POLICARD (1936) and others have attributed the circulation of nutrient fluids to the resiliency of the cartilage; application of a load compresses the cartilage which at once expands to its original shape when the load is removed. This is equivalent to a kind of pumping action in the cartilage, the fluid being drawn in and again, more or less completely, expressed. If the resiliency of the cartilage is impaired, the pumping action and hence the circulation will become less effective. INGELMARK & EKHOLM (1948) have demonstrated that human as well as rabbit articular cartilage is thicker immediately after a few minutes' physiological joint exercise, the swelling rapidly subsiding during periods of rest. The most probable explanation of this is that the articular cartilage first imbibes and subsequently expels fluid. EKHOLM & NORBÄCK (1951) reported that after brief joint exercise the articular cartilage exhibits histological changes probably signifying alterations of chondral fluid content.

Apart from purely mechanical factors that are capable of facilitating the nutrition of articular cartilage, the physical and chemical nature of the intercellular matrix must be taken into consideration. The degree of chondroitin sulphate polymerization would thus seem to be of importance, at least for the rate of diffusion. The degree of polymerization is probably dependent on age, activity and severity of pathological lesions (GERSH & CATCHPOLE, 1949).

Here it may be appropriate to recall the reaction to enzymatic action of the ground substance in various tissues, for example

the depolymerizing action of hyaluronidase on its substrate. A reduction of chondroitin sulphate viscosity (which is precisely what hyaluronidase action produces) should facilitate and accelerate the exchange of fluids with the environment.

Metabolism of Articular Cartilage

According to BAUER, ROPES & WAINE (1940), the respiratory quotient of articular cartilage is low. The fact that no chondrocytes come into immediate contact with arterial blood, they pointed out, implies that glycogen is decomposed through anaerobic oxidation. LUTWAK-MANN (1940) showed that articular cartilage is practically incapable of oxidizing some substances rapidly oxidized by other tissues. Having studied experimentally the metabolism of bovine articular cartilage, ROSENTHAL, BOWIE & WAGONER (1941) found that from infancy to old age the chondrocyte content decreases 75 per cent, that the glycolysis rate is proportional to the chondrocyte content whatever the age, and that the oxygen consumption diminishes at a higher rate than the chondrocyte content. In other words the metabolism of articular cartilage slows down with advancing age.

The chondroitin sulphate metabolism will be discussed in Part II.

Growth and Regenerative Ability of Articular Cartilage

ELLIOTT (1936), among others, studied the normal growth mechanism of articular cartilage from young and adult rabbits, rats, cats and dogs. From his findings he drew the following conclusions. Firstly, mitosis was the normal mechanism of cell division in immature cartilage. It became rarer as maturity was approached and could not be demonstrated in the adult even allowing for diurnal rhythm and rapidity of fixation and after adequate exercise. Secondly, as mitosis fell off amitotic figures appeared and amitosis became the sole normal method of maintenance growth in the adult. Thirdly, in both types of

growth maximum activity occurred a short distance beneath the surface and fell off abruptly outwards and gradually inwards. Fourthly, proliferative activity seemed to be conditioned by two factors acting from the articular surfaces, one inhibitory and one stimulatory. Both factors decreased with distance from the surface, the inhibitory faster than the stimulatory.

Studying the normal attrition of articular cartilage, HAMMAR (1894) found in the synovial fluid numerous formed elements derived from both the synovial membrane and the cartilage. For a long time his results were sadly neglected by other investigators, but HULTÉN & GELLERSTEDT (1940/41) later rediscovered them. They studied the decomposition and resorption in joints of abraded cartilage particles. Their opinion was that the articular cartilage is normally being abraded and that discrete cartilage particles sometimes are torn out. Indeed, formed elements, probably abraded cartilage and synovial membrane particles, have been found in normal synovial fluid but they are more plentiful after joint exercise (EKHOLM & NORBÄCK, 1951).

This continuous attrition of the articular cartilage must obviously be compensated by regeneration. Available evidence indicates that the regeneration mainly results from chondrocyte proliferation in the intermediate stratum. Thus SILBERBERG (1936) observed proliferation of the chondrocytes in the intermediate stratum of articular cartilage from guinea pigs given anterior pituitary extract. The phenomenon, first reported by HOLMDAHL & INGELMARK (1948), that prolonged excessive exercise produces a rearrangement of articular cartilage structure has also been investigated by SÄÄF (1950). He found that most of the new chondrocytes required for the regenerative process come into being through proliferation in the intermediate stratum.

The reaction of articular cartilage to injuries created operatively or otherwise has long been a subject commanding the interest of researchers. The old view was that articular cartilage is incapable of regeneration. An apt illustration of this is the following excerpt from HUNTER's (1743) writings: »From Hippocrates to the present age it is universally allowed that ulcerated cartilage is a troublesome disease and that when once destroyed

it is not recovered.» The literature from more modern times includes a large number of papers describing experimental studies on articular cartilage repair. In all papers I know of that describe the reaction of articular cartilage due to experimentally induced defects the number of test animals has been rather small, most investigators having used series of 10 to 15 animals. Observation periods exceeding 6 to 7 months are exceptional and in most cases they have not even been that long. Moreover, selective staining techniques have seldom been used and the attention paid to the ground substance has been far too cursory in these experimental investigations. Lastly, regeneration originating in the chondrocytes within the articular cartilage itself has not always been distinguished from that due to the mesenchymal cells in the subchondral marrow spaces.

Judging by the literature, only SEGGEL (1904) and FASOLI (1905) have observed — after 12 and 9 days respectively — mitoses in (rabbit) articular cartilage with experimentally induced defects.

One paper only compares young and old animals with respect to the repair of articular cartilage defects. The exception to the rule is BENNETT & BAUER's (1935) investigation using puppies and dogs. The repair of cartilage defects in their experiments was »no more rapid or complete in young puppies before closure of the epiphysis than it is in adult dogs».

LEIDY (1849), KÖLLIKER (1852), PAGET (1853), GURLT (1853), GUSSENBAUER (1871) and other contemporary authors maintained that hyaline cartilage lacks regenerative ability. However, as these older experimental investigations were made before the advent of aseptic techniques, the results may have been influenced by infection. This is not borne out by GIES' (1882) interpretation of his own findings: cartilage defects in aseptic joints did not repair while those in infected joints did. Here the use of carbolic acid to prevent infection might have inhibited the regenerative process. Among later investigators may be mentioned CIOCIOLA (1921) and HÄBLER (1925) who arrived at the conclusion that articular cartilage has no regenerative ability.

The first report of a spontaneously repaired defect in articular cartilage came from REDFERN (1851). He incised articular cartilages from dogs and found that the defect would heal through the formation of fibrous tissue at the site of the injury. Cell proliferation commenced at the margins of the incision which was filled with »a granular mass, containing very imperfect fibres, and a number of corpuscles not to be distinguished from the nuclei of cartilage cells». When the articular cartilage had been incised obliquely, REDFERN found structures he called polynucleated cartilage cells below the superficial cells of the area surrounding the scar.

Among more modern researchers with the opinion that articular cartilage is capable of regenerating, mention may be made of SHANDS JR (1931) who recapitulated the more significant results obtained up to that time. From his own experiments on canine articular cartilage he concluded that regeneration occurs, that it is at its peak after 12 weeks, and that it is manifested by the appearance of large new polynucleated chondrocytes. He found regeneration of hyaline cartilage in superficial defects not involving the subchondral bone. The following quotation from SHANDS' paper refers to a group of defects most of which penetrated to the subchondral bone; the first group examined after 12 weeks comprised 10 defects in 3 joints, the second group examined after 8 weeks 10 defects in 3 joints, and the third group examined after 4 weeks 21 defects in 4 joints: »There are definite evidences of new cartilage cells in 70 per cent of the twelve week sections, 60 per cent of the eight week sections, and 10 per cent of the four week sections.» Scrutiny of SHANDS' photographic material reveals that the regenerated tissue in no case filled the defect completely.

In experiments on dogs BENNETT, BAUER & MADDOCK (1932) found that »histological evidence of repair of cartilage by proliferation of cartilage cells was present in four of the six defects which were entirely within cartilage. In no instance was repair complete or perfect within the twenty-eight week period.» After mentioning that there appeared »clusters of regenerating cartilage cells», they stated: »The proliferative

activity of cartilage cells is greater in the deeper zones of articular cartilage than in the more superficial zones. The most satisfactory repair was observed in the two defects in which 'protected' crevices existed.»

Distinguishing between the regenerative activity in the central and that in the peripheral regions of the articular cartilage, many researchers maintain that the regenerative ability is far lower centrally (FISHER, 1922—23, 1939; BENNETT, BAUER & MADDOCK, 1932). FISHER (1922—23, 1939) expressed the opinion that, owing to the nutritional differences between the central superficial areas and the peripheral area of the articular cartilage, it was not surprising »to find a difference in response of these different zones to both injury and disease». In one series of rabbits he removed the central articular cartilage down into the bone and prepared sections after 22 weeks; in another series incisions were made in the central and peripheral parts of the articular cartilage, sections being prepared after varying lengths of time. In the first of these series removal of the central poorly nourished area of the articular cartilage was followed by compensatory proliferation of the peripheral better-nourished areas. In this connection FISHER surmised that the marginal lipping (chondroosteophytes) seen in osteoarthritis might be a manifestation of compensatory proliferation in the peripheral areas of the articular cartilage following destruction by injury or disease of the central areas. FISHER's second series yielded the following results. Incisions in the central portion remained unhealed for long periods while incisions in the peripheral parts healed more rapidly and there was a formation of new cartilage by active proliferation of chondrocytes. This has been borne out by BENNETT, BAUER & MADDOCK (1932) who also found that the central chondrocytes had a lower proliferative activity than the peripheral.

The reaction that follows superficial defects involving only the articular cartilage is to be distinguished from the response after injuries which penetrate the subchondral bone. Several researchers have stated that if the subjacent bone is injured so that the bone marrow spaces are opened, the marrow often

produces granulation tissue. In such cases metaplasia of mesenchymal cells coming from the bone marrow turns them into chondrocytes and fibrocartilage is formed (TIZZONI, 1878; FASOLI, 1905; RIMANN, 1905; FISHER, 1922—23; ITO, 1924—25; HÄBLER, 1925; SHANDS JR, 1931; BENNETT, BAUER & MADDOCK, 1932). It has been observed that the greatest regenerative activity occurs after such deep injuries which involve the subchondral bone (SHANDS JR, 1931; BENNETT, BAUER & MADDOCK, 1932).

A number of workers have observed that the regenerative activity in response to operative defects in articular cartilage is accompanied by the development of degenerative changes in the cartilage. FASOLI (1905), for example, described degenerative alterations in the vicinity of the lesion. AXHAUSEN (1912, 1923), BURCKHARDT (1924) and HÄBLER (1925) have all observed degeneration or osteoarthritic changes following articular cartilage defects created operatively or otherwise. Unsuccessful attempts to reproduce these findings have been made by various authors including BENNETT, BAUER & MADDOCK (1932). KEY (1933 B) found degenerative changes with cytoclasis after partial removal of articular cartilage. When he removed relatively large portions of the cartilage from the femoral condyle in rabbit, severe degeneration (»well marked arthritic-like changes») ensued in over half the cases. On the other hand, if only a superficial cartilage layer was pared off, slight degeneration (»mild arthritic changes») was produced in about half the cases. He emphasized that a degree of cytoclasis always occurs in the neighbourhood of an injury in articular cartilage. In another investigation KEY (1933 A) demonstrated that the vulnerability to traumatization, such as by chemical action following repeated intraarticular injections of mild acids and alkalis, and the reduction of proliferative activity were greatest for chondrocytes in the deeper layers of articular cartilage. The conclusion he drew from these observations was that articular cartilage grows from beneath rather than from the surface and that the deeper cells are younger than those nearer the surface.

CHAPTER III

MATERIALS AND METHODS

Materials

Apparently healthy rabbits of both sexes were used as test animals in the experiments to be described. There was one group of animals up to 3 months old and another with adult animals. The average weight of the young animals was 1.8 kg and that of the adults 3.2 kg. They were adequately fed with turnips, hay and oats. Each animal had its own cage with a floor area of approximately 0.3 m^2. The number of animals included in the different experiments appears in Table 1.

TABLE 1.

Number of rabbits with operatively created patellary cartilage defects classified according to age, supplementary treatment and period of postoperative survival time, the average for Period III being 9.4 months.

Age	Other Treatment	Period I (< 3 mos.)	Period II (3–6 mos.)	Period III (> 6 mos.)
Adult	None	27	33	20
Adult	Plastic Film Applied	4	8	6
Young	None	8	7	6

Controls

Apart from the control material available in the form of the Institute of Anatomy's histological slides with sections from normal untreated rabbit knee joints, a series of control slides was prepared especially for the present investigation. For this purpose adult rabbit knee joints were arthrotomized parapatellarly either by a single medial incision, or by

both a medial and a lateral insicision, the patellary cartilage being left intact. Moreover, for selective staining, pieces of patellary and femorocondylar cartilage were excised from the knees of normal adult rabbits.

Methods

Operative Techniques

Basal anaesthesia was induced preoperatively with 3 ml of 25 per cent urethane solution per kg body weight, the operation itself being done under ether. Routine aseptic precautions were observed. The area to be operated on was depilated and washed with 10 per cent benzalkonium chloride solution (»Desivon» brand). After a medial parapatellary incision had exposed the patella it was rotated about its longitudinal axis far enough to make the cartilage surface accessible. With the aid of a small and very sharp scalpel a thin chip was now pared off from the central part of the patellary cartilage. Care was taken not to injure the marginal portions. At most one fourth of the patellary cartilage area was removed and the cut in no case penetrated to the subchondral bone.

In most cases both hindlegs of the animal were operated on. The excised cartilage pieces were examined histologically to determine the depth of the cut. The deepest point of the cut was situated about half way into the radiate stratum. After the patella had been replaced the joint capsule and fascia were sutured in apposition and the skin separately with catgut No. 3/0. The skin wounds were dusted with sulpha-penicillin powder and painted with liquid plastic. Immediately after the operation and the next day the rabbits were given single penicillin doses of about 10.000 I.U. by the subcutaneous route. Neither sulpha drugs nor antibiotics were applied intraarticularly.

In 24 knee joints the entire surface of the defective patellary cartilage was covered with a 0.2 mm thick polyethylene film which was sutured with silk or catgut to the fascia in front of the patella. Application of the polyethylene film made it necessary to incise both sides of the joints.

After the operation the animals were given the freedom of

their cages for periods ranging from 1 week to 22 months (cf. Table 1), before they were killed by a sharp blow on the back of the head.

Complications

Occasional deaths occurred under anaesthesia or a few days postoperatively; the knee joints of 3 animals presented gross signs of infection; laterad luxation of the patella had taken place in 2 joints. All these animals were excluded when the results were analyzed and none are included in Table 1.

Post Mortem Procedures

All the patellae, and from occasional joints femorocondylar cartilage pieces also, were removed at autopsy. Small cartilage pieces intended for selective staining were removed from the edge of the operative defect on the freshly prepared and yet unsectioned patellae from some 40 rabbits altogether. All the patellae were fixed in formaline, decalcified in 10 per cent formic acid solution, dehydrated, paraffine embedded, and sectioned. The sections, 6 μ thick, were cut normal to the free cartilage surface and parallel with the patellary major axis. Sometimes the whole patella was serially sectioned; in all other cases every 5th to 10th section were saved. It was thus possible to obtain a general idea of the histological appearance of all parts of the patellary cartilage.

Staining methods. Sections from all the decalcified patellae were routinely stained with haematoxylin-eosin.

The following selective staining methods were used on sections cut from the aforementioned pieces of patellary and femorocondylar cartilage. Lison's method of metachromatic staining with toluidine blue after fixation in basic lead acetate was applied to patellary and femorocondylar cartilage from 42 cartilages. Romeis' (1948) method of staining for fats with scarlet red or Sudan III was used on frozen sections of patellary and femorocondylar cartilage from 27 cartilages. Glycogen, manifested by Romeis' method for Best's carmine stain, was estimated in patellary and femorocondylar cartilage from 22 cartilages.

CHAPTER IV

MORPHOLOGICAL FINDINGS

A. Clinical Observations

During the first 24 hours after the operation the animals lay still most of the time. As little as 2—3 days after the operation they seemed to move about in their cages without much difficulty and ate normally. A week after the operation the skin wounds had healed. During the first week the knee joints usually showed symptoms of intraarticular exudation, but 8—10 days postoperatively there was no swelling and the volitional knee mobility seemed the same as before the operation.

B. Gross Morphological Findings

On all the defective patellary cartilages the site of the primary lesion was visible to the naked eye. This was so even when a long time (about a year) had elapsed after the operation (Figs. 2 and 3). Even after long periods the size, depth and rough corrugated floor of the operative defect remained the same as when it was made. Only the edges of the defect tended gradually to stand out less sharply against the surroundings. The uninjured parts of the patellary cartilage and the other cartilages in the joint appeared normal. The volume of the synovial fluid was not noticeably increased. Osteoarthritis-like changes were seen only in the presence of luxations and infections and occasionally when the patella had been covered with plastic.

C. Histological Findings

In this section are described findings made in 146 patellary cartilages from adult animals and in 36 patellary cartilages

from young animals. Unless otherwise stated the descriptions refer to observations made in cartilages from adult animals; a fairly large proportion of the illustrations refers to young patellary cartilages and two plastic-covered cartilages because the changes observed therein often happened to be more characterisitic.

Histological examination revealed that a number of changes had taken place after the operation in most of the patellary cartilages. These changes were evident not only in the immediate neighbourhood of the central operative defect but also in the other parts of the cartilage. Single or aggregated cells were encountered which have no counterparts in corresponding sections from normal patellary cartilage. The appearance, location in the cartilage or frequency of these formations disclosed that they were composed of new cells. Hence it was considered justified to reckon such formations as signs of regeneration. Here it is interesting to note that neither among these new cells nor among normal chondrocytes were mitoses ever observed. In addition to regeneration, the patellary cartilages with operative defects often exhibited alterations with features long recognized as typical signs of degeneration. Classified according to their nature, postoperative changes of the following types were observed in the patellary cartilages.

Regenerative Changes

a. Atypical chondrons. A collection of chondrocytes or chondrocytoid cells forming an aggregate that deviates with respect to cell quantity and configuration from chondrons in the corresponding strata of untreated patellary cartilage.

Atypical chondrons occured in two varieties, hereinafter to be known as variant A and variant B, which in this series seldom coexisted.

Atypical chondrons variant A, the commonest in both young and adult cartilages, consisted of spheroid or ellipsoid aggregates of closely packed cells (Figs. 4, 5 and 6). The number of cells per chondron was often very large, so large indeed that some such formations could aptly be described as giant chondrons. The cells, of spheroid or ellipsoid shape and frequently vacuo-

lated, had pale cytoplasm and were morphologically undistinguishable from normal chondrocytes. In the neighbourhood of variant A chondrons there were often other chondrons with the appearance characteristic of that stratum, and the surrounding tissue itself seemed fairly normal.

Haematoxylin-eosin staining revealed the variant A chondrons circumscribed by a dark coloured zone outside which there was intercellular matrix of lighter hue and homogeneous structure. Selective staining disclosed that the cells of the variant A chondrons contained about as much fat as normal chondrocytes. Glycogen was also demonstrated in these cells although the stain was less distinct and the quantity lower than in normal chondrocytes. Almost invariably toluidine blue imparted a sharply metachromatic colouration to the immediate neighbourhood of the variant A chondrons (Fig. 7).

Atypical chondrons of variant A occured at all levels in the patellary cartilage except in the superficial stratum. They were largest and most plentiful in the intermediate stratum and outer radiate stratum. Some were seen also in the deeper parts of the radiate stratum, particularly when this was the only stratum remaining after the operation (Fig. 8). Variant A formations could even develop in partially detached cartilage shavings (Fig. 9).

Variant A chondrons could be found as little as a month after the operation in both young and adult animals, but they became more common longer time after the intervention.

Atypical chondrons of variant B were generally composed of cells smaller than normal chondrocytes and of spheroid or polygonal shape. The nucleus was somewhat flattened and the cytoplasm appeared dark in sections stained with haematoxylin-eosin. The cells did not look like fully differentiated, mature chondrocytes. Variant B chondrons resembled variant A chondrons in shape, size and cell number. Examples of really gigantic variant B chondrons were observed (Figs. 10 and 11).

Unfortunately none of the undecalcified cartilage pieces subjected to selective staining happened to be taken from any of the few patellary cartilages in which B chondrons were

observed. Therefore, considering that the adopted methods of staining for fats and glycogen cannot be applied to decalcified sections, it could not be determined whether these substances were present in variant B chondron cells. Normal chondrons with characteristics typical of the cartilage stratum in question were seldom observed in the neighbourhood of variant B chondrons. Most variant B chondrons were — unlike variant A chondrons — surrounded by an abundance of pale coloured intercellular matrix. Toluidine blue always produced pronounced metachromasia in the immediate neighbourhood of B-atypical chondrons. (Fig. 12)

Chondrons of variant B occured in the intermediate and radiate strata but not the superficial stratum of the patellary cartilage. In one caze they had the appearance of a hood on a bone marrow canal penetrating the calcified cartilage stratum, which suggests that connections might exist between the cells in such variant B chondrons and the bone marrow cells (Fig. 13).

Variant B chondrons were first seen 2 months (1 adult cartilage) and occurred most frequently more than 5 months postoperatively (*i.e.* in Periods II and III of Table 1. p. 24). Variant B chondrons were about as common in patellary cartilages from adult and from young rabbits.

Atypical chondrons were observed in 81 of altogether 146 adult patellary cartilages; variant A chondrons in 75 (about 90 %) and variant B chondrons in 8 (about 10 %) of these 81 cartilages. Both types of atypical chondrons coexisted in only 2 units. Variant A occurred in 30 of 36 cartilages from young rabbits, variant B in a single such unit which had been plastic covered.

The atypical chondrons were found exclusively in the peripheral parts — that is remote from the central defect — of approximately 75 per cent of the 83 patellary cartilages characterized by chondron atypicality. About 25 per cent of these cartilages had atypical chondrons exclusively in the central parts below or near defect, and only one cartilage exhibited atypical chondrons both centrally and peripherally.

b. Cell swarms. In 7 patellary cartilages from adult and 3

from young rabbits swarm-like conglomerations of spheroid or polygonal, propinquent, immature chondrocytes were seen extending practically from top to bottom of the cartilage. There was very little, moderately metachromatic intercellular matrix. Most of these cell swarms had visible connections with the bone marrow (Fig. 14). These cell swarms were observed in cartilages from both young and adult rabbits, the earlier being 2 months postoperatively in one adult cartilage and as late as 5 months after the operation in young cartilages.

c. Cell islands. Islands of closely spaced, small, fibrocytoid cells were observed at or close to the site of the defect on 10 adult and 2 young patellary cartilages (Figs. 15 and 16). The sparse intercellular matrix showed no metachromasia. These cell islands occurred as isolated formations centrally on the defect and, as verified by examination of consecutive serial sections, had no connections whatever with the subchondral marrow spaces or the marginal cartilage regions. The islands were anything up to 20 cell layers thick but never thick enough to reach the level the cartilage surface would have had if left intact. They were first observed 1 month postoperatively in one young patellary cartilage and 2 months postoperatively in two adult cartilages, the others being encountered not less than 4 months after the operation.

d. Restored superficial stratum. The site of the operative defect on one young and one adult patellary cartilage was found to be covered with a structure essentially identical to the superficial stratum of an intact cartilage (Fig. 17). The amount and appearance of the intercellular matrix were normal. One restored superficial stratum was found 5 months (the adult) and one year after the operation (the young).

Degenerative Changes

Examination of haematoxylin-eosin stained sections revealed that practically all the patellary cartilages with operative defects showed impaired stainability of both nuclei and cytoplasm in the chondrocytes belonging to the first two or three cell layers immediately below the surface of the defect. This phenomenon

became manifest fairly early but seldom less than a month after the operation.

Sections from comparatively many patellary cartilages contained large areas where reduced stainability and nuclear pyknosis characterized the chondrocytes (Fig. 18). Occasionally the cartilage structure was eradicated more or less completely: cells and nuclei were unstainable, or ghost cells only appeared, or a structureless light eosin coloured mass was all that remained of the cartilage, whose surface then might exhibit crevice formation (Fig. 19).

Selective staining of cartilage from regions with impaired chondrocyte stainability revealed that such chondrocytes contained little or no substance stainable with scarlet red or Sudan III, that is fatty compounds. Similarly, such chondrocytes proved deficient in or devoid of glycogen. In addition, in areas with imperfectly staining or unstainable chondrocytes in haematoxylin-eosin sections the intercellular matrix exhibited respectively impaired or abolished metachromasia.

The changes described in the three preceding paragraphs are undoubtedly degenerative in nature. However, owing to the difficulty of estimating degrees of degeneration in haematoxylin-eosin stained sections, it was thought advisable to distinguish only two grades of cartilage destruction: hereinafter called incomplete and complete degeneration.

For the purposes of the present investigation, incomplete cartilage degeneration will be defined by diminished haematoxylin-eosin stainability of cells and intercellular matrix, the chondrocytes containing little or no fats and glycogen. Similarly, complete cartilage degeneration is characterized by almost eradicated cartilage structure with at best ghost cells persisting, by fibrillation and crevice formation on the surface, and by practically no metachromasia. Here incomplete and complete are purely qualitative terms and have no quantitative connotations whatsoever.

The degree of cartilage degeneration manifested by limited areas of diminished stainability of two or three chondrocyte layers was not even recorded as incomplete degeneration;

involvement of at least 4 to 5 cell layers was considered a minimum requirement.

Both degrees of degeneration occurred about equally often in young and adult patellary cartilages. Incomplete cartilage degeneration often appeared as early as some 6 weeks after the operation, but complete cartilage degeneration usually required at least 4 months to develop.

Changes satisfying the above criteria were recorded in 65 adult patellary cartilages, degeneration being incomplete in 61 units (93 %) and complete in 4 units (7 %). The two grades of degeneration occurred together in 4 patellary cartilages. Among young cartilages 16 showed incomplete and one complete degeneration.

Degeneration, whether incomplete or complete, was predominantly peripheral in one-third and predominantly central in two-thirds of the patellary cartilages classified as degenerated (60 units). Both peripheral and central degeneration occurred in all cartilage strata, sometimes in more than one stratum of the same cartilage. Marginal degeneration often extended from the surface half way into the radiate stratum.

CHAPTER V

CONTROLS AND PATELLARY CARTILAGES COVERED WITH PLASTIC FILM

Controls

None of the forms of regeneration recognized in the foregoing were observed in patellary cartilage sections from normal rabbits. Nor have, so far as I am aware, such formations been described in the literature on normal articular cartilage from rabbit.

Neither regenerative nor degenerative changes were encountered in patellary cartilage sections from animals arthrotomized but not patellotomized 3 months previously.

Fat globules were observed in practically all the chondrocytes in patellary and femorocondylar cartilage from normal young and adult rabbits (Fig. 20). In all strata these fat globules were a little smaller and in the superficial stratum perhaps more frequent in patellary cartilages from young than in those from adult animals. No definitely fat-staining substance was present intercellularly.

Glycogen was present in practically all chondrocytes of normal rabbit patellary cartilage. It was present most abundantly in the cytoplasm of the larger chondrocytes in the radiate and intermediate strata but could be observed also in the small flat chondrocytes in the superficial stratum. Cartilages from young and adult rabbits displayed no differences in glycogen content.

Everywhere but in the calcified stratum of normal rabbit patellary cartilage sections stained with toluidine blue the intercellular matrix was markedly metachromatic. Unlike the corresponding human tissue (HIRSCH, 1944), also the superficial stratum of the rabbit patellary cartilage contained meta-

chromatic material, although the metachromatic colouration of the outermost layers of this stratum occasionally was rather faint in sections obtained from both young and adult animals.

Patellary Cartilages Covered with Plastic Film

The operative defect on 23 patellary cartilages from 18 adult rabbits had been covered with a polyethylene film. Almost without exception this plastic film was perforated so that only ragged shreds remained around the margins of the cartilage whenever the animals were allowed to live more than 3 months after the operation. As chemical action by the synovial fluid was considered most unlikely, this indicates that the plastic film was subjected to considerable friction.

Fourteen of 23 cartilages presented regenerative changes in the form of atypical chondrons (variant A only) and in one cartilage a cell island was observed. In 22 of these cartilages degenerative changes were present, the degeneration being complete in 8 units. This indicates that the frequency of regeneration did not alter appreciably when the operative defect was covered with plastic whereas the frequency of degeneration tended to increase compared with animals not so treated.

CHAPTER VI

STATISTICAL ANALYSIS

Introduction

146 cartilages from 80 adult animals, namely 74 from right and 72 from left legs were analysed statistically. It was deemed necessary to make separate analyses for the two sides as one animal's two patellary cartilages probably would not react independently.

The right and, separately, the left patellary cartilages were classified with respect to the time elapsed from operation to autopsy, the resulting groups being subdivided according to the character of the reaction. It was decided to distinguish between short (Period I < 3 months), medium (Period II $3-6$ months) and long (Period III > 6 months) survival times, the average for Period III being 9.4 months. The character of the reaction was simplified to the following categories: »regeneration + degeneration», »regeneration only», »degeneration only», and »no changes». Presence of atypical chondrons of variant A or variant B was made the sole criterion for regeneration. Cell swarms were repudiated because they were not believed to originate from the patellary chondrocytes, cell islands because of their doubtful origin, and restored superficial strata because they were so few. The criterion for degeneration was taken to be presence of degeneration, incomplete and/or complete, as defined on p. 32.

The results of this chapter will be found on p. 47.

For full descriptions of the statistical methods used in this work standard textbooks should be consulted. Here it may be mentioned that percentages were tested by the Chi-square

distribution, means by analysis of variance or the t-distribution, and correlation coefficients by Fisher's z-transformation. χ^2-values and other data were obtained from PEARSON & HARTLEY's (1954) »*Biometrika, Tables*».

Abbreviations

s = standard deviation
r = correlation coefficient
F = F-distribution
χ^2 = Chi-square distribution
t = t-distribution
df = degrees of freedom
p = probability

Levels of Significance

Not significant		$p > 0.1$
Possibly significant	0.1	$\geq p > 0.05$
Significant on 5 per cent level	0.05	$\geq p > 0.01$
Significant on 1 per cent level	0.01	$\geq p > 0.001$
Significant on 0.1 per cent level	0.001	$\geq p$

Basic Classification of Patellary Units

Table 2 shows the classification of patellary units.

TABLE 2.

Number of right and left patellary cartilages presenting various changes within periods of postoperative survival time.

Character of Changes	Period I (< 3 mos.)		Period II (3–6 mos.)		Period III (> 6 mos.)	
	Right	Left	Right	Left	Right	Left
Regeneration & Degeneration.	4	4	9	8	8	8
Regeneration Only	4	4	12	11	6	5
Degeneration Only	5	6	4	4	4	1
No Changes	12	10	4	7	2	4
Total	25	24	29	30	20	18

Time and Regeneration

The relationship between the length of time elapsed from operation to autopsy — the same periods of postoperative survival time as in Table 2 — and the frequency of regeneration was evaluated by dividing the patellary cartilages into two categories. One of these groups included units presenting regeneration, alone or with degeneration; the other comprised units displaying degeneration only or no changes whatsoever. The justification for this classification principle is that regeneration may be regarded as an active tissue process whether it is accompanied by degeneration or not, whereas absence of changes and degeneration represent respectively non-activity and retrogression. The results of this classification are presented in Tables 3, 4 and 5.

TABLE 3.

Number and frequency in per cent of right patellary cartilages presenting regeneration, alone or with degeneration, and degeneration only or no changes, within periods of postoperative survival time.

Mode of Reaction	No. of Units				Frequency, %			
	Period			Total	Period			Total
	I	II	III		I	II	III	
Regeneration only or Regeneration & Degeneration	8	21	14	43	32	72	70	58
Degeneration only or No Changes	17	8	6	31	68	28	30	42
Total	25	29	20	74	100	100	100	100
$\chi^2 = 13.1$	$df = 2$			$p < 0.01$				

The value of χ^2 in Table 3 indicates that in periods II and III the frequency of regeneration was higher than in period I, the difference being significant on the 1 per cent level.

TABLE 4.

Number and frequency in per cent of left patellary cartilages presenting regeneration, alone or with degeneration, and degeneration only or no changes, within periods of postoperative survival time.

Mode of Reaction	No. of Units				Frequency, %			
	Period			Total	Period			Total
	I	II	III		I	II	III	
Regeneration only or Regeneration & Degeneration	8	19	13	40	33	63	72	56
Degeneration only or No Changes	16	11	5	32	67	37	28	44
Total	24	30	18	72	100	100	100	100
$\chi^2 = 6.46$	$df = 2$			$p < 0.05$				

The value of χ^2 in Table 4 indicates that in periods II and III the frequency of regeneration was higher than in period I, the difference being significant on the 5 per cent level.

TABLE 5.

Number and frequency in per cent of combined right and left patellary cartilages presenting regeneration, alone or with degeneration, and degeneration only or no changes, within periods of postoperative survival time.

Mode of Reaction	No. of Units				Frequency, %			
	Period			Total	Period			Total
	I	II	III		I	II	III	
Regeneration only or Regeneration & Degeneration	16	40	27	83	33	68	71	57
Degeneration only or No Changes	33	19	11	63	67	32	29	43
Total	49	59	38	146	100	100	100	100
$\chi^2 = 19.6$	$df = 4$			$p < 0.001$				

The value of χ^2 in Table 5, which is the sum of 13,1 and 6,46, indicates that in periods II and III the frequency of regeneration was higher than in period I, the difference being significant on the 0.1 per cent level. The greater part of the difference is due to the increase in the frequency of regeneration which took place from period I to period II.

Time and Degeneration

Here, too, the patellary cartilages were divided into two categories: one group with cartilages presenting degeneration, whether or not accompanied by regeneration; the other group comprising all the rest, that is those displaying regeneration and those without changes. When the patellary cartilages in Table 2 are thus classified, Tables 6, 7 and 8 are obtained.

TABLE 6.

Number and frequency in per cent of right patellary cartilages presenting degeneration, alone or with regeneration, and regeneration only or no changes, within periods of postoperative survival time.

Mode of Reaction	No. of Units				Frequency, %			
	Period			Total	Period			Total
	I	II	III		I	II	III	
Degeneration alone or Degeneration & Regeneration ..	9	13	12	34	36	45	60	46
Regeneration only or No Changes	16	16	8	40	64	55	40	54
Total	25	29	20	74	100	100	100	100
$\chi^2 = 2.60$	$df = 2$			$p > 0.1$				

The value of χ^2 in Table 6 indicates that the several periods showed no significant differences in the frequency of degeneration.

TABLE 7.

Number and frequency in per cent of left patellary cartilages presenting degeneration, alone or with regeneration, and regeneration only or no changes, within periods of postoperative survival time.

Mode of Reaction	No. of Units				Frequency, %			
	Period			Total	Period			Total
	I	II	III		I	II	III	
Degeneration alone or Degeneration & Regeneration ..	10	12	9	31	42	40	50	43
Regeneration only or No Changes	14	18	9	41	58	60	50	57
Total	24	30	18	72	100	100	100	100
$\chi^2 = 0.45$	$df = 2$			$p > 0.1$				

The value of χ^2 in Table 7 indicates that the several periods showed no significant differences in the frequency of degeneration.

TABLE 8.

Number and frequency in per cent of combined right and left patellary cartilages presenting degeneration, alone or with regeneration, and regeneration only or no changes, within periods of postoperative survival time.

Mode of Reaction	No. of Units				Frequency, %			
	Period			Total	Period			Total
	I	II	III		I	II	III	
Degeneration alone or Degeneration & Regeneration ..	19	25	21	65	39	42	55	45
Regeneration only or No Changes	30	34	17	81	61	58	45	55
Total	49	59	38	146	100	100	100	100
$\chi^2 = 3.05$	$df = 4$			$p > 0.1$				

The value of χ^2 in Table 8, which is the sum of 2,60 and 0,45,

indicates that the several periods showed no significant differences in the frequency of degeneration. The mean frequency of degeneration in the whole series of patellary cartilages was about 45 per cent.

Time and Regeneration versus Degeneration

As it has been found that the frequency of degeneration was substantially the same in the three periods, a mean frequency of degeneration for all the patellary cartilages of 45 per cent will be used in the following, the greater number of units tending to make the statistical evaluation more reliable. The statistical analysis will be based on the frequencies given in Tables 3 and 4 as well as 6 and 7.

Period I. In this period the combined right and left patellary cartilages showed a regeneration frequency of about 33 per cent while the corresponding degeneration frequency was about 45 per cent. After χ^2-analysis of each side's patellary cartilages, the χ^2 values thus obtained were combined, giving: $\chi^2 = 2.2$, $df = 2$, $p > 0.1$. This value of χ^2 indicates that in period I the frequency of regeneration was not significantly different from the frequency of degeneration.

Periods II and III. In this combination period the total right and left patellary cartilages showed a regeneration frequency of 69 per cent and a degeneration frequency of 45 per cent. Just as for period I, the results of unilateral χ^2-analysis were combined, giving: $\chi^2 = 14.2$, $df = 2$, $p < 0.001$. This value of χ^2 indicates that in the combination period (II + III) the frequency of regeneration showed a predominance significant on the 0.1 per cent level over the frequency of degeneration.

Association of Regeneration and Degeneration in Patellary Units

In order to establish whether an association, direct or inverse, existed between regeneration and degeneration, the time factor

was eliminated and all the right patellary cartilages were treated as one group and all the left units as another group. These groups were then subdivided with respect to the mode of reaction. Now both regeneration and degeneration can theoretically be manifested in two ways: presence of that mode of reaction (reaction) or absence of the other mode of reaction (0 reaction). Consequently there are four possibilities, namely regeneration (reaction), degeneration (0 reaction) = a unit presenting regeneration only; degeneration (reaction), regeneration (0 reaction) = a unit presenting degeneration only; regeneration (reaction), degeneration (reaction) = a unit presenting regeneration + degeneration; and regeneration (0 reaction), degeneration (0 reaction) = a unit presenting neither regeneration nor degeneration. The patellary cartilages in Table 2 have been thus classified in Tables 9 and 10.

TABLE 9.

Number of right patellary cartilages presenting neither, either or both of the two modes of reaction, regeneration as well as degeneration being considered either present (reaction) or absent (0 reaction). See above.

Regeneration / Degeneration	0 Reaction	Reaction	Total
0 Reaction	18	22	40
Reaction	13	21	34
Total	31	43	74
$\chi^2 = 0.345$	$df = 1$	$p > 0.1$	

The value of χ^2 in Table 9 indicates that in the right patellary cartilages regeneration and degeneration were not associated and occurred independently of one another.

TABLE 10.

Number of left patellary cartilages presenting neither, either or both of the two modes of reaction, regeneration as well as degeneration being considered either present (reaction) or absent (0 reaction). Cf. p. 43.

Regeneration / Degeneration	0 Reaction	Reaction	Total
0 Reaction	21	20	41
Reaction	11	20	31
Total	32	40	72
$\chi^2 = 1.77$	$df = 1$	$p > 0.1$	

The value of χ^2 in Table 10 indicates that in the left patellary cartilages regeneration and degeneration, were not associated and occurred independently of one another.

Accordingly, as appears from Tables 9 and 10, regeneration and degeneration were not associated and occurred independently of one another in the patellary cartilages when these were regarded as integral units.

Association of Regeneration and Degeneration in Patellary Zones

The result of the preceding section does not preclude the existence of an association, direct or inverse, between regeneration and degeneration locally in the patellary units. These *units* were regarded as consisting of three parallel layers as defined in the key to Tables 11 and 12. According to a simple extension of the principle adopted in the preceding section, the figures designated as Table 11 and Table 12 were compiled from the original notes recording the sites of the two modes of reaction as observed in the patellary cartilages included in Table 2. Certain simplifications were made in the subsequent analysis. Units presenting neither regeneration nor degeneration were rejected as being useless for evaluating an association between these modes of reaction. The association was arbitrarily considered strong when no zone presented a single mode of

TABLE 11.

Right Patellary Cartilages

Degeneration	Regeneration O	T	M	B	TM	TB	MB	TMB
O	18	4	6	2	3		5	2
T	9	2	1		1			
M			2					
B	2			1				
TM			3		1		1	
TB						1		
MB	1			1	1		1	
TMB	1	1		1			2	1

TABLE 12.

Left Patellary Cartilages

Degeneration	Regeneration O	T	M	B	TM	TB	MB	TMB
O	21	3	6	3	6		2	
T	3		1	1				
M	3							
B	2			1			2	
TM	1		2					
TB						1		
MB	1			2				
TMB	1	2	2	1	1		2	2

Key to Tables 11 and 12.

The patellary cartilages are thought of as divided into three layers of approximately equal thickness known as the top (T), middle (M) and bottom (B) zones. O = no reaction, T = reaction in top zone (superficial and intermediate strata), M = reaction in middle zone (outer radiate stratum), B = reaction in bottom zone (deep radiate stratum), TM = reaction in top and middle zones, TB = reaction in top and bottom zones, MB = reaction in middle and bottom zones, and TMB = reaction in top, middle and bottom zones.

reaction — such units will be found along the diagonal from the top left to the bottom right corners of the figures. The association was arbitrarily considered moderate when one or two zones presented both modes of reaction and either mode was present in an additional zone — in the figures such units have any of the letters *T*, *M* or *B* common to regeneration and degeneration. It was considered that no association existed when regeneration and degeneration nowhere coexisted, that is had none of the letters *T*, *M* or *B* in common.

In Tables 13 and 14 the column for observed units is derived from the corresponding figure, Tables 11 and 12 respectively, and the column for expected units is the calculated random distribution.

TABLE 13.

Observed and expected random zonal distributions of different strengths of association between regeneration and degeneration in right patellary cartilages presenting either or both modes of reaction.

Degree of Association	Distribution	
	Observed	Expected Random
Strong	9	6.2
Moderate	11	26.7
None	36	23.1
Total	56	56.0
$\chi^2 = 27.9$ $df = 2$	$p < 0.001$	

The numerical analysis and χ^2 value in Table 13 reveal that a difference, significant on the 0.1 per cent level, existed between the observed and expected random distributions. Most of this large difference is accounted for by the fact that the number of units actually presenting moderate association was significantly lower than if the distribution had been random.

The result of the corresponding analysis on the left patellary cartilages appears in Table 14.

TABLE 14.

Observed and expected random zonal distributions of different strengths of association between regeneration and degeneration in left patellary cartilages *presenting either or both modes of reaction.*

Degree of Association	Distribution	
	Observed	Expected Random
Strong	4	5.7
Moderate	14	24.3
None	33	21.0
Total	51	51.0
$\chi^2 = 12.7$ $df = 2$	$p < 0.01$	

The numerical analysis and χ^2 value in Table 14 reveal that a difference, significant on the 1 per cent level, existed between the observed and expected random distributions.

The results obtained from Tables 13 and 14 imply that no direct association existed between regeneration and degeneration in the various zones of the patellary cartilage. Indeed, the two modes of reaction were inversely associated. In other words, when degeneration exists in a particular zone of the patellary cartilage, the probability of also encountering regeneration in that zone is reduced.

Results

The frequency of regeneration increased with time, the preponderance of period II and III over period I being significant on the 0.1 per cent level. Most of the difference is accounted for by the increased frequency of regeneration in period II compared with period I. The frequency of regeneration was 33 per cent in period I, 68 per cent in period II and 71 per cent in period III.

The various periods did not differ significantly in frequency of degeneration. In the series as a whole the frequency of degeneration was 45 per cent.

In period I the frequency of regeneration and that of degeneration did not differ significantly, but in periods II and III regeneration predominated over degeneration, the difference being significant on the 0.1 per cent level.

In the patellary cartilage as a whole regeneration and degeneration were not associated and occurred together quite independently of one another. However, when the patellary cartilages were considered as consisting of three layers,[1]) each with neither, both or either mode of reaction, it was possible to obtain an expression for the predominant mode of reaction per cartilage. Analysis of these figures disclosed that regeneration and degeneration were dissociated, that is the difference between observed and expected (random) predominance was significant on the 0,1 per cent level. In other words, regeneration would be more likely to appear in layers without than in layers with prior degeneration.

[1]) The layers are defined in the key to Tables 11 and 12.

CHAPTER VII

DISCUSSION AND CONCLUSIONS

The literature does not, so far as I am aware, record any case of gross healing of shallow defects made operatively in the centre of articular cartilages from experimental animals. The present investigation confirms this — operative defects inflicted upon rabbit patellary cartilages presented no gross evidence of healing, not even if more than one year had elapsed after the intervention.

Among previous investigators who have made experimental studies on the reparative or regenerative capability of articular cartilage following operative interventions, the large majority have created deep defects extending to or penetrating the subchondral bone and only a few have concerned themselves with shallow defects. Since the aim of the present investigation was to determine the nature and degree of any regenerative capability intrinsic to, and the general response of, the articular (patellary) cartilage, shallow defects only were created in the central parts of the cartilage. Microscopical examination at various times after the operation revealed that the patellary cartilage sections displayed either regenerative changes or degenerative changes or regenerative and degenerative changes or no histologically manifest changes.

Regenerative Changes

The concensus of previous investigators' opinion is that one form of regeneration is evidenced by the development of large chondrons composed of numerous chondrocytes, although these formations have variously been described as »giant chondrons» (WEICHSELBAUM, 1872), »proliferation of cartilage cells» (SEGGEL,

1904), »large cell nests» (KEY, 1931, 1933 A) and »clusters of cartilage cells» (BENNETT, BAUER & MADDOCK, 1932). In the same class must probably be reckoned what some authors have interpreted as multinucleated cartilage cells (PENNISI, 1904; FISHER, 1922—23; AXHAUSEN, 1923; HÄBLER, 1925; SHANDS JR, 1931). For, judging by the latter authors' illustrations, their multinucleated cells could more appropriately be described as chondrons composed of many chondrocytes whose nuclei are markedly predominant over the cytoplasm. The atypical chondrons — of which two variants, A and B, were distinguished — observed in the present investigation would seem to conform to most if not all of these previously reported regenerative formations, the majority of them being equivalent to variant A.

Being observed in 75 of 146 adult and in 30 of 36 young patellary cartilages with operative defects, atypical variant A chondrons constituted by far the most frequent form of regenerative activity. Variant A chondrons probably arose through proliferation of the chondrocytes in the patellary cartilage itself, and the cell division may have been triggered by the irritation due to the operation. The likelihood of this mode of development is borne out by the facts that atypical chondrons of variant A were encountered fairly close to the articular surface of the patellary cartilage and in semidetached cartilage shavings — that is remote from the mesenchymal cells in the subchondral marrow spaces — and that examination of consecutive serial sections revealed no connections between any variant A chondrons and the cells of the marrow spaces. If, as seems likely, KEY's (1931) »large cell nests» are equivalent to variant A chondrons, it is interesting to consider a subsequent paper by KEY (1933 A) where he demonstrated that the development of apparently identical formations could be triggered also by non-operative traumatization such as that due to repeated intraarticular injections of weak acids or alkalis.

In the previous literature it is difficult to find an unchallengeable counterpart to the present investigation's atypical chondrons of variant B, as observed in only 8 adult

patellary cartilages with operative defects. In adult cartilages atypical variant B chondrons may have arisen in the same way as variant A chondrons, although the chondrocytes failed to differentiate after dividing. For three reasons it seems rather unlikely that variant B chondrons were precursors of variant A. First, almost invariably the first variant B chondrons were encountered somewhat longer after the intervention than the first variant A chondrons; about 18 per cent of the variant A chondrons and some 12 per cent of the variant B chondrons occurred in Period I. Second, variant B chondrons were seldom observed in any patellary cartilage with variant A. Third, almost always the cartilage surrounding variant B chondrons was degenerated and rather seldom that surrounding variant A chondrons. Another possibility is that atypical chondrons of variant B might have originated from mesenchymal cells in the preformed canals through the calcified stratum. Such a mode of development is suggested by the fact that in one patellary cartilage an atypical variant B chondron was seen sitting as a hood atop a bone marrow canal, with the cells of the chondron apparently attached to the cells in the canal.

Regenerative changes characterized by the formation of what has been described as, for example, »fibrocartilage» (SHANDS JR, 1931) or »connective tissue containing new cartilage cells» (BENNETT, BAUER & MADDOCK, 1932) apparently conform to the cell swarms noted in the present study. Encountered in 7 adult and 3 young patellary cartilages, these cell swarms had the appearance of large masses, sometimes extending through all the cartilage strata, of small, immature chondrocytes in close proximity whose intercellular matrix contained metachromatic material. The latter observation suggests that these cells were related more closely to chondrocytes than to fibrocytes. Presumably, on the evidence of consecutive serial sections, these cell swarms were derived from the mesenchymal cells in the bone marrow spaces, notwithstanding the fact that the primary injury far from reached the subchondral bone, let alone penetrated it. This may in fact be a corollary to the initial stage of osteoarthritis when, according to LLOYD-

ROBERTS (1953), mesenchymal cells pass through the subchondral bone lamella and grow upwards into the articular cartilage. The present investigation accordingly suggests that proliferation of new chondrocytes can take place from the mesenchymal cells in the bone marrow spaces even when the defect in the cartilage fails to reach the subchondral bone.

A form of regeneration which has been described as a connective tissue pannus containing spindle cells on the surface of the defect (SHANDS JR, 1931) has its counterpart in the cell islands seen in 10 adult and 2 young cartilages in the present investigation. These formations had the appearance of islands of aggregated fibrocytoid cells, whose intercellular matrix contained no metachromatic material, which were situated on the surface of the defect in the patellary cartilages. Examination of consecutive serial sections disclosed no connections coming to these cell islands from the periphery of the patellary cartilage where the tucked in portion of the synovial membrane was attached or from the mesenchymal cells in the bone marrow. Conceivably, therefore, the cell islands — which so as to speak floated on the surface of the defect — may have arisen through division of chondrocytes which stopped differentiating at an early stage. SHANDS JR (1931) made no statements regarding the mode of development of such formations.

The defective area of one young and one adult patellary cartilage in the present series was partially covered by a new superficial stratum. The literature does not, so far as I am aware, describe anything like this type of regeneration; but then, as it is so rare, it cannot be of much practical importance. The restored superficial stratum never covered the defective area completely, and the thickness of the cartilage never became appreciably greater than immediately after the defect had been created.

The majority of authors, among them FISHER (1922—23, 1939) and BENNETT, BAUER & MADDOCK (1932), have found the strongest regenerative activity peripherally and the weakest centrally in the articular cartilage. These authors created defects both centrally and peripherally, however, and so their

results are not directly comparable with my findings. In the present investigation it was found that, among the 83 of 146 adult patellary cartilages where regeneration in the form of atypical chondrons of either variant had developed subsequent to central lesions, about one-fourth presented central regeneration and about three-fourths peripheral regeneration, that is fairly remote from the actual lesion in the latter case. The fact that the regenerative activity was lower in the central parts of the cartilage even though the defect was created therein may perhaps be attributed to poorer nutritional conditions for the central than for the peripheral parts of the patellary cartilage. From this it can be argued that wherever the defect is made, indeed even when it is located centrally, the seemingly better nourished peripheral parts of the patellary cartilage respond by showing compensatory regeneration. When an operative defect has been created centrally in the patellary cartilage, the remaining peripheral portions will have to support a greater load. Now it is not unlikely that these peripheral portions strive to make up for this by bracing themselves to withstand this greater load and that the development of atypical chondrons marginally in the cartilage is a sign of such reinforcement.

Various authors including OGSTON (1875—76), SILBERBERG (1936), ELLIOTT (1936) and SÄÄF (1950) have stated that regenerative growth of articular cartilage, at least such growth as compensates for normal attrition, takes place predominantly in the intermediate stratum. Not so FISHER (1939), who regarded the superficial chondrocytes as mother cells for the fully developed chondrocytes in the deeper parts of the cartilage. Nor KEY (1933 A), whose opinion was quite the opposite. He declared that articular cartilage grows from beneath rather than from the surface and the deeper cells are younger than those nearer the surface. Former authors have not considered the intermediate stratum particularly important as a regenerative zone in the repair of operative defects in articular cartilage. CIOCIOLA (1921) and SHANDS JR (1931) instead emphasized that the highest regenerative activity was obtained after defects involving the subchondral bone. BENNETT & BAUER (1935)

found that the chondrocytes showed a higher susceptibility to proliferate in the deep than in the outer strata of articular cartilages from puppies. In the present investigation no particular stratum of the patellary cartilage was predominant with respect to proliferative activity of the chondrocytes, although the chondrocytes in the intermediate or superficial radiate strata might have tended to proliferate slightly more than the chondrocytes situated in the deep layers of the radiate stratum, at any rate with respect to the development of atypical chondrons of variant A.

This result bears out rather than contradicts previous investigators' view that the intermediate stratum is a zone of regeneration. On the other hand, no evidence was obtained in support of BENNETT & BAUER's (1935) opinion that the deeper strata of articular cartilage with operative defects are responsible for the highest regenerative activity.

Having created a number of operative defects in various joints of each of 14 dogs, SHANDS JR (1931) recorded regeneration in response to 60 per cent of the lesions 8 weeks after the operation and to 70 per cent 12 weeks after the operation. BENNETT, BAUER & MADDOCK (1932) observed chondrocyte proliferation in response to 4 of 6 shallow defects not involving the subchondral bone, but the latter authors omitted to mention how long after the operation the animals were sacrificed. In my investigation the patellary cartilages were classified in three groups with respect to the animal's postoperative survival time, namely less than 3 months (Period I), 3 to 6 months (Period II) and over 6 months (Period III), the average for Period III being 9.4 months. From a series of 80 adult, rabbits classified under these three periods 146 patellary cartilages with lesions going about half way into the radiate stratum were obtained. Only the findings made in these cartilages were analyzed statistically. It appeared that the frequency of regeneration was significantly higher in Period II (68 %) than in Period I (33 %) and showed no additional increases in Period III (71 %). As these percentages are based on a more comprehensive series of adult rabbits — 146

patellary cartilages — their reliability must be proportionately greater than the corresponding figures published by previous authors.

Degenerative Changes

Most previous researchers have paid little or no attention to the degenerative changes accompanying operative defects in the articular cartilage. Yet both FISHER (1922—23) and KEY (1931) reported that mild degeneration almost always sets in beneath operative defects. KEY (1931) emphasized that operations on articular cartilage were accompanied by pronounced degenerative and even »arthritic-like» changes. (It seems that KEY referred to those hypertrophic changes which ultimately arose following his operations on articular cartilage.) FISHER (1922—23) found cartilage necrosis after operations down to the calcified stratum. BENNETT, BAUER & MADDOCK (1932), on the other hand, expressed the opinion that osteoarthritic changes would not develop unless luxations were present. PAYR (1923) and HÄBLER (1925) both remarked that operations on joint cartilage would not be accompanied by osteoarthritis so long as the joint remained functionally unimpaired.

The surface of the defect on all the patellary cartilages in my series showed mild degeneration to a depth of one or two cell layers. Quite often, however, the degeneration was more extensive and complete degeneration of the cartilage also occured. FISHER's (1922—23) and KEY's (1931) observations of degenerative changes in articular cartilage following operative interventions thereon are compatible with my findings, although none of the patellary cartilages in my series presented true osteoarthritic alterations. In the present investigation joint function was never restricted: the animals were not immobilized in plaster casts but given the freedom of their cages throughout the postoperative survival period. Accordingly there is nothing to prove or disprove PAYR's (1923) and HÄBLER's (1925) statements to the effect that maintenace of joint function tends to prevent the onset of osteoarthritis secondary to operations on the articular cartilage.

In the present series of patellary cartilages, degenerative changes were encountered in all strata, somewhat more frequently in the superficial stratum and also beneath and around the margins of the defect. According to KEY (1933 A and B), on the other hand, the most pronounced degenerative reaction subsequent to operative or other traumatization occurred in the deep strata of the articular cartilage.

Among those patellary cartilages from my series with changes recorded as degeneration (65 of 146 adult and 17 of 36 young units) — that is degeneration extending beyond the immediate vicinity of the defect and penetrating deeper than two or three cell layers — about two-thirds exhibited degeneration in the central portions and about one-third showed such changes in the peripheral portions of the patellary cartilage.

KEY (1933 B) reported that mild »arthritic-like» changes were encountered in 50 per cent of rabbit femoral cartilages from which the superficial stratum had been removed at a preceding operation. He omitted to specify the number of cartilages operated on however. In my series of 146 patellary cartilages which was subjected to statistical analysis, it appeared that the frequency of degeneration remained practically constant at 45 per cent in the series as a whole and also for each of the three periods. These figures are not directly comparable with KEY's (1933 A), however, for in my investigation the changes had the appearance of more or less complete cellular degeneration rather than of »arthritis». Nevertheless, judging by KEY's (1933 A) illustrations, the changes observed by him were more characteristic of osteoarthritis than of arthritis. If such is the case, one might say that his findings and mine are in fairly good agreement.

Particularly in the older literature, it has often been stated that the presence of fat in chondrocytes is a sign of their degenerating. In my investigation the opposite was the case — whereas the chondrocytes in degenerated cartilage contained little or no fat, normal cartilage practically always exhibited an abundance of intracellular fat. This bears out MONTAGNA's

(1944) contention that fat accumulation intracellularly is not necessarily a degenerative process.

Association between Regenerative and Degenerative Changes

Whether, and if so how, regeneration and degeneration are associated is a problem that seems to have escaped the attention of investigators of articular cartilage responses to operative defects. In the present study it was found, as mentioned, that different regions of the patellary cartilage differed in their susceptibilities to regeneration and degeneration. It will be remembered that regeneration predominated in the marginal and degeneration in the central parts of the patellary cartilage. While the main reason for these deviations no doubt was the fact that the defect was made centrally in the cartilage, a contributory factor might have been the apparently poorer nutrition of the central than of the peripheral parts of the patellary cartilage.

As noted, the frequency of degeneration in adult cartilages remained practically constant at 45 per cent in all three periods. Hence, in the first period with survival times up to 3 months, the frequency of regeneration did not differ significantly from that of degeneration. On the other hand in the two subsequent periods with survival times in excess of 3 months, the regeneration frequency was significantly higher than the degeneration frequency. It is rather remarkable that the frequency of degeneration showed no appreciable variation with lengthening survival times after the operation; on the other hand, it may merely reflect the poor regenerative ability of articular cartilage.

When the patellary cartilage was considered as a unit and the zonal distribution of the respective changes was neglected, regenerative and degenerative changes appeared in no way associated. On the other hand, when the patellary cartilage was divided into three zones and the existence of neither, either or both regeneration and degeneration was recorded for each zone separately, regeneration seemed to be associated with absence of degeneration. In other words, regenerative changes

are more likely to occur in a zone without than in a zone with degenerative processes. This seems to be in good agreement with what one would expect from biological points of view. KEY (1933 A and B) seems to attach great importance to the primary degeneration as a triggering factor for the regenerative activity. No such conclusions can be drawn from my findings which indicate that degeneration need not precede regeneration at the site in question. Moreover, comparisons are not possible because, so far as I am aware, no investigations have been made into the relationship between degeneration and regeneration in different layers of the articular cartilage in osteoarthritic conditions.

It is interesting to consider the articular cartilage's response following operative defects and the degenerative or regenerative changes occasionally seen in osteoarthritis. Thus, HULTÉN & GELLERSTEDT (1940—41) maintained that incipient cartilage degeneration is coexistent with regenerative growth. In osteoarthritic conditions attended by the development of regenerative and degenerative changes, these processes often assume truly abnormal proportions and may even cause mechanical impairment of joint function, as for example when pronounced marginal lipping is present. Operative defects on articular cartilage give rise only to slight regeneration and in the rabbit do not lead to any measurable diminution of joint mobility. Furthermore, the regenerative changes secondary to an operation need not be associated with simultaneous degenerative changes within the articular cartilage, as always would seem to be the case in osteoarthritic states. Consequently the nature and mode of development of the regenerative changes secondary to osteoarthritis do not seem to be analogous with the nature and mode of development of the regenerative changes occurring in response to operative defects.

Regenerative changes were observed in 30 and degenerative in 17 of 36 young patellary cartilages. The corresponding figures for adult patellary cartilages were 81, 65 and 146. Hence the frequency of regeneration seemed higher in young than in adult cartilages. BENNETT & BAUER (1935), on the other

hand, found no such difference between young and adult patellary cartilages.

Protection of the operative defect with a polyethylene film displayed no tendency to enhance the frequency of regeneration; conversely, it tended to increase the frequency of degeneration. This observation indicates that it seems unwise to let plastic articulate against cartilage following joint operations. Most of the polyethylene films used to cover the operative defect were more or less worn out after three months, an observation indicating the magnitude of the frictional forces acting on two articulating surfaces in a joint that is used.

PART II

AUTORADIOGRAPHIC STUDIES

CHAPTER VIII

SURVEY OF THE LITERATURE

DZIEWIATKOWSKI, BENESCH & BENESCH (1949) found a high concentration of S^{35} in articular cartilage from 7 days old rats, after intraperitoneal administration of S^{35}-labelled Na_2SO_4. They suggested that most, if not all, the labelled sulphate sulphur retained by the cartilage might be retained therein as chondroitin sulphate. Other authors, among them BOSTRÖM (1953), concur in this view.

On the other hand, as shown by experiments on albino rats (BOSTRÖM & ÅQVIST, 1952), S^{35} is not incorporated to any great extent in thio-amino acids. These authors found that the S^{35}-incorporation was very high in the sulphate group of the chondroitin sulphuric acid in the cartilage while it was very low — only one thirtieth as high — in taurine and practically non-existent in cystine and methionine. The maximum uptake in chondroitin sulphuric acid and taurine was reached in 24 and 8 hours respectively. On these grounds, administration of S^{35}-labelled sulphate has been called a more or less specific method of »staining» for sulphomucopolysaccharides (ODEBLAD & BOSTRÖM, 1952; BOSTRÖM, 1953).

Given to rats in the form of sodium sulphate, S^{35} is rapidly excreted in the urine and faeces, hardly any demonstrable S^{35} being left in the blood after 120 hours (DZIEWIATKOWSKI, 1949 A). When S^{35} is injected into the blood stream, the total blood sulphur radioactivity reaches a maximum in 2 hours and declines to 8 per cent of the peak value 24 hours after the injection (BOSTRÖM, 1952). Accordingly, since considerable amounts of S^{35} are accumulated in cartilage even though the substance is rapidly being excreted from the body, the rate of S^{35} uptake in cartilage must be fairly rapid. BOSTRÖM (1952) administered

S^{35}-labelled sodium sulphate to adult rats and at intervals from 2 hours to 16 days after the injection isolated chondroitin sulphate from the costal cartilage. By thus following the uptake and elimination of S^{35}, he found that the amount taken up culminated 24 hours after the injection and then slowly declined to half the maximum 16 days after the injection. BOSTRÖM (1953) stated that, provided samples are taken more than 24 to 48 hours after injection of the isotope, good agreement is found in the cartilage between the fluctuations in the relative amount of S^{35}, as estimated by quantitative autoradiography, and the variations of S^{35} in the ester sulphate derived from isolated chondroitin sulphuric acid whose radioactivity has been measured with a Geiger-Müller counter.

It is nowadays known that the S^{35} uptake is a direct measure of the total chondroitin sulphate synthesis. RODÉN (1956), after reviewing a number of investigations carried out up to then, has perhaps formulated this in the best manner: »Through the work of BOSTRÖM (1952, 1953) it was unequivocally shown that S^{35}-sulphate was fixed in cartilage as a part of chondroitin sulphuric acid, which was obtained in a high degree of purity. BOSTRÖM also studied the turnover rate of this polysaccharide in rats following injections of S^{35}-sulphate. The results of such in vivo studies on the incorporation of S^{35}-sulphate in mucopolysaccharides can most probably be interpreted as an expression of the metabolic activity of the whole molecule. Such a view is supported by the recent demonstration of SCHILLER et al. (1955) that the various parts of the carbon skeleton and the sulphate group of chondroitin sulphuric acid turn over at approximately the same rate in skin.» It should be noted that the results of the latter investigation apply specifically to chondroitin sulphuric acid from skin, but most probably they are equally applicable to chondroitin sulphuric acid derived from cartilage.

Although rigorous proof is lacking, the chondroitin sulphate synthesis is believed to take place in the chondrocytes (SYLVÉN, 1947; BELANGER, 1954; etc.).

By experiments on calf costal cartilage cut into relatively

thick slices BOSTRÖM & MÅNSSON (1953) demonstrated that the incorporation of S^{35} into chondroitin sulphuric acid was dependent on the presence of intact vital chondrocytes. When the chondrocytes were killed by heat or subdivision, the chondroitin sulphuric acid was rendered incapable of incorporating S^{35}. The authors found much evidence showing that S^{35} uptake is a process under enzymatic control, the sulphur-metabolism stimulating factor probably being glutamine (BOSTRÖM, RODÉN & WESTERMARK, 1955).

Studying growing epiphyseal rat cartilage, BELANGER (1954) established the close association between the S^{35} uptake and the chondrocytes, particularly the most active and vital ones. He showed that the chondrocytes initially incorporate S^{35}, but after 36 hours part of this S^{35} has been transferred to the intercellular matrix, and after 6 days all the labelled sulphate lay outside the chondrocytes. ODEBLAD & NORHAGEN (personal communication; submitted for publication 1957) found both 24 and 48 hours after the radiosulphate injection chondrocytes in bronchial and tracheal cartilage whose autoradiographic image appeared as a white spot surrounded by a black ring. BELANGER indirectly emphasized the significance for S^{35} uptake of cell vitality by remarking that »in the area of degenerated cartilage where the cells are dead or dying, no autoradiograph has been recorded.» This obviously implies that these »dead or dying» cells had incorporated no S^{35}.

Some authors have studied the association between metachromasia and S^{35} uptake of cartilage. BELANGER (1954), for example, found that the two phenomena were on the whole parallel. A high S^{35} uptake in the chondrocytes seemed to be associated with pronounced metachromasia of the intercellular matrix in the same region of the cartilage. DUTHIE & BARKER (1955) were unable to corroborate this. They used epiphyseal and articular rat cartilage sections. Yet, although all their sections were stained for metachromasia with toluidine blue, they could not relate intensity of colouration to degree of radioactivity in any area.

CHAPTER IX

MATERIALS AND METHODS

Materials

One young and 32 adult rabbits fed and caged as described in Part I, p. 25 were used. Table 15 shows the number of animals involved in each of the five experiments. The total number of animals in Table 15 exceeds 33 because most of them were utilized for more than one experiment.

TABLE 15.

Experiment	No. of animals	Subject Studied
1	22	S^{35} Distribution Between Cells and Intercellular Matrix in Articular cartilage at Different Times After the Injection
2	15	S^{35} Distribution in Articular Cartilage Strata
3	11	S^{35} Uptake of Operated and Unoperated Articular Cartilage A. Autoradiography B. Photometry C. Cell Density in Operated and Unoperated Articular Cartilage
4	10	A. Correlation Between Total Area and Density of Cells and S^{35} Uptake of Operated Articular Cartilage
	11	B. Correlation Between Density of Cells and S^{35} Uptake of Unoperated Articular Cartilage
5	10	Relationship Between S^{35} Uptake and Metachromasia in Articular Cartilage

Methods

S^{35} Administration. Operative Procedures

S^{35} was selected for the present autoradiographic investigation owing to its β-radiation of low penetration (particle energy = 0.167 MeV) and its convenient half-life of 87.1 days.

Carrier-free, S^{35}-labelled sodium sulphate in aqueous solution (pH 7) was injected intravenously in a dosage of 1 mC per kg body weight, except where otherwise stated. Supplied by Messrs. L. K. B. Produkter, Stockholm, the radiosulphate was produced by the Atomic Energy Research Establishment, Harwell, England. After the injection the animals were given the freedom of their cages for periods ranging from 7 hours to 24 days.

Sufficiently long after the operation (cf. Table 16) to recover from its immediate effects, the animals were given the S^{35} injection. The animals were operated as described in Part I and sacrificed by a blow on the head.

TABLE 16.

No. of animals	Treatment
17	Unilateral removal from central patellary cartilage of chip extending half way into radiate stratum.[1])
5	Bilateral removal from central patellary cartilage of chip extending half way into radiate stratum.[2])
2	Unilateral arthrotomy only
2	Unilateral arthrotomy only + contralateral removal of cartilage chip
7	None

[1]) One operated joint was injected with 5 per cent formalin solution in order to produce degenerative changes.

[2]) Plastic film was also applied over four patellae in order to produce degenerative changes.

Preparative Techniques

Immediately after the animals had been killed the knee joints were opened, the patellae excised and pieces removed

from that portion of the femorocondylar cartilage which articulates against the patellary cartilage. Cartilage pieces were also taken from the floor and immediate vicinity of the defect on the operated and from the corresponding parts of the intact patellary cartilages. Both the femorocondylar and the patellary cartilage pieces went down to the calcified stratum.

The excised cartilage pieces were at once immersed in methanol for 24 hours, transferred to propanol for 3 hours and then embedded in paraffin. Fixation in methanol and propanol with subsequent paraffin embedding precludes dissolution by water-containing solutions later entering the cartilage so effectively that the use of this method permits (ODEBLAD, 1952) the assumption that any radiosulphur incorporated in the mucopolysaccharides will be retained therein rather than being dissolved at some point in the preparation procedure. The embedded cartilage pieces were cut into 10 μ sections which were mounted on slides and deparaffined with xylene and ethanol; at this time no stains were applied.

For photometric purposes a section thickness of 10 μ was found to be the best compromise between conflicting requirements. To reduce the untoward effects of secondary radiation, absorption and transit distance the sections should be as thin as possible, and to permit minimal doses of radiosulphate and yet obtain adequate blackening of the film they should be as thick as possible. In this case section thicknesses below 10 μ produced inadequate blackening when the S^{35} dosage was imited to 1 mC per kg body weight.

Autoradiographic Methods

Autoradiographs were recorded by the contact method and also by the stripping film technique.

Contact autoradiography. The sections used for recording contact autoradiographs were mounted on 1 mm thick methacrylic slides: glass would probably have shattered if brought into intimate contact with the film in a screw press or with clamps, as required for the method described by ODEBLAD (1952) and adopted here.

Gevaert Dentus Rapid film was used. This is a coarse-grained X-ray film of great sensitivity and low resolving power. The film was exposed for 9 days, except in experiments 3 and 4 B where the exposure time was 6 days. It was developed for 15 minutes at 19° C in Johnson's fine grain developer. After fixation, rinsing and drying, the emulsion was rubbed off from the reverse side of the doubly coated film base with a rag moistened in a potassium bichromate+sodium thiosulphate solution.

The contact autoradiographs were examined under the microscope and compared with the sections from which they had been recorded after these had been histologically stained with haematoxylin-eosin or toluidine blue.

Stripping film autoradiographs. These were recorded as described by PELC (1947) on Kodak autoradiographic film. This is a fine-grained film which gives satisfactory resolution but requires excessive exposure times. Thus, in my experiments, adequate autoradiographs could not be recorded in less than about 2 months. These films were developed for 30 minutes at 19° C in Kodak D 19 B developer.

As the stripping film autoradiographs adhered to the cartilage sections, microscopical examination permitted more accurate collation of blackening to particular cells than was possible with contact autoradiographs.

Resolving power of film emulsions. According to data published by GROSS, BOGOROCH, NADLER & LEBLOND (1951), the maximal resolution obtainable with Gevaert Dentus Rapid film is of the order of 12 μ. In my experiments the resolution averaged about 20 μ, as measured on a photomicrograph.

Under favourable conditions stripping film permits a maximal resolution of about 2.5 μ (DOMIACH & PELC, 1950), but it was estimated that the maximal resolution achieved in my experiments was some 10 μ.

Determination of Cell Density and Total Cell Area

The cells in suitable strips were counted under a microscope fitted with an eye-piece square-net micrometer (in the following

referred to as e. s. m.). Such a strip formed a rectangle with the short side parallel to the joint surface of the cartilage and two squares (160 μ) of the e. s. m. wide. The long side of the rectangle passed right across the cartilage section.

The nuclei rather than the cells were counted. The calculation was performed by means of an e. s. m. and 240 times magnification. Cells whose nuclear calottes had a diameter smaller than 2 μ were disregarded. Nor were the nuclei counted in clearly degenerated cells (as these incorporate no S^{35}, cf. p. 00). The nuclei were counted within the entire region selected, i. e. the previously described strip with a short side of 160 μ. Then the average number of nuclei per unit area of the section, i. e. 80×80 μ was calculated.

The total cell area was determined by photomicrographing various portions of the histological section with the e. s. m. applied. The negatives were enlarged by an equal amount and copied on thin paper. In the selected strips cells having nuclear calotte diameters exceeding about 2 μ were then cut out and weighed on an analytical balance. The total cell area is proportional to the weight of the »paper cells». The weight of paper per unit section area was calculated and correlated with the degree of blackening of the corresponding autoradiograph portion.

Metachromatic Staining

The relationship between radiosulphate uptake and metachromatic staining of the cartilage ground substance was determined by examining sections of patellary and femorocondylar cartilage from normal and operated knee-joints. After contact autoradiographs had been recorded, the sections were stained with toluidine blue according to Lisson's technique. The resulting metachromasia was examined under the microscope and classified as follows: Nil (0), weak (+), moderate (++), and strong (+++). This classification was related to the corresponding blackening of the contact autoradiographs.

As shown by PERSSON's (1953) investigations it is most important to use dyes of great purity for metachromatic stai-

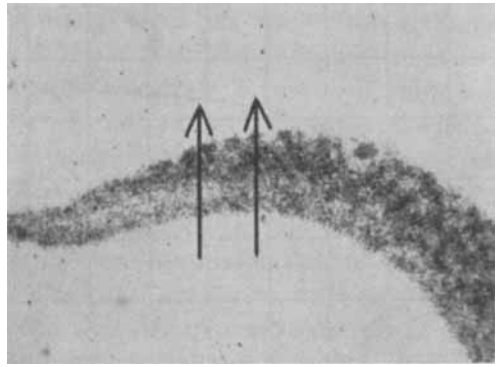

Fig. 21. Autoradiograph recorded from patellary cartilage section. The direction of the paths traversed by the photometer aperture is shown by the arrows.

ning. In the present investigation the same proprietary preparations and the same stock solutions were used throughout.

Microphotometry

Contact autoradiographs were photometered by a modification of the method described by ODEBLAD (1952, 1956). The microphotometer[1]) (designed and constructed by BOURGHARDT, BRATTGÅRD, HYDÉN, JIEWERTZ & LARSSON, 1953) with a motorized feed table moving the film at a constant velocity past the objective of a microscope while a recorder draws a curve proportional to the intensity of the transmitted light. The accuracy of the photometer itself is better than 1 % (BOURGHARDT & coworkers, 1953). The standard error of the photometric proecdure is 15—20 per cent of the total mean (i. e. the variation coefficient is 15—20 per cent). This figure includes all errors of method in experiment 3. The computation is based on the residuals in the analysis of variance in Tables 19, 20 and 21. The photometered autoradiographs were always traversed in a direction normal to the free joint surface of the cartilage section (Fig. 21). The magnification was such that

[1]) Through the courtesy of Professor H. HYDÉN the instrument at the Department of Histology, University of Gothenburg, was made available.

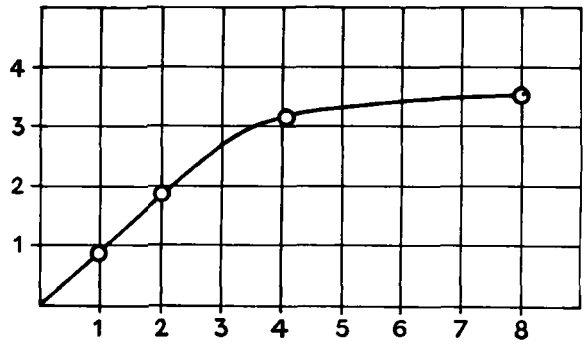

Fig. 22. Reference curve. Seconds of exposure are marked off along the abscissa and photometer readings (= relative amount of S^{35}) along the ordinate. Photometer readings obtained for exposures of 1, 2, 4 and 8 seconds are ringed. New reference curve was made each time the photometer was used.

the aperture for the photomultiplier tube scanned a 15 μ wide strip of the autoradiograph.

The gain and zero level of the photometer amplifier driving the recording mA-meter were adjusted with the aid of a reference standard. This was made by letting a constant source of faint light expose Gevaert Dentus Rapid film for 0, 1, 2, 4, and 8 seconds. This standard film was photometered and the excursions of the pen were adjusted with the gain control so that the width of the paper was optimally utilized. The photometer readings thus obtained were plotted against exposure time in a Cartesian system of coordinates. The time in seconds corresponding to a given mA-reading-and hence degree of blackness — could thus be read off directly. Hence the blackening of the autoradiographs was expressed in arbitrary (relative) units (R. U.) based on seconds (Fig. 22).

Photometric curves for determining the correlation between degree of blackening and cell density or total cell area per unit section area (experiment 4) were recorded for strips of differing cell characteristics. These strips in the histological sections were selected so that they could easily be matched with the corresponding strips of the contact autoradiographs, i. e. close to irregularities or crevices which could be indentified in the autoradiographs.

The corresponding strip on the autoradiograph was found and traversed four times with the photometer whose effective aperture diameter was 15 μ. After each photometric recording the feed table was moved sideways about 2 aperture diameters. Each selected strip was thus photometered over a combined rectangular area 60 μ wide. As the short side of the strip was 160 μ wide, the average of the smoothed curve values should provide a fairly close approximation to the average blackness of the strip.

When it was necessary to estimate the average blackening of an autoradiograph or a selected rectangle, a number of photometer curves were traced for each autoradiograph or rectangle. In order to obtain an average for each curve, it was smoothed by drawing a line approximately half way between the peaks (the blackening of details) and valleys of the curve and measuring the distance from this line to the zero level. As a rule the line could be drawn free hand. To check the validity of this smoothing technique 22 curves were accurately measured by planimeter and by dividing the area under the curve into several narrow columns and calculating their mean height above the zero level. The same curves were then measured by the smoothing technique. When the mean for the smoothing technique was compared with the mean for the planimeter method and the mean for the »columnmeasuring» procedure, the respective differences turned out to be -0.03 ± 0.06 and -0.07 ± 0.10. Neither of these differences are significant, so I considered the free-hand smoothing technique sufficiently precise.

Relationship between Autoradiographic Blackening and S^{35} Uptake. ODEBLAD (1952) showed that the curves for P^{32} exposure and light exposure were almost parallel. As both S^{35} and P^{32} radiate only β-particles it is reasonable to believe that the curves for S^{35} and light are parallel also. The blackening curve for light is not linear on any film and becomes almost parallel with the time axis with long exposures. However, the photometer was always adjusted to operate within that part of the reference curve making an angle of about 45° with the zero line where the relationship between photometer readings and

time (seconds) was fairly linear. The arbitrary values on the seconds scale could thus be used to measure the relative amount of radiosulphur incorporated in the traversed part of the section.

The degree of blackening is dependent upon the relative radiosulphur amount existing under a given area of the contact autoradiograph.

The radiation intensity which emanates from this area of the section determines the blackening of a corresponding area of the autoradiograph. The recorded radioactivity comes from the entire volume of the section under the area concerned. Thus, a summation effect of all radioactive particles within this volume will be obtained because the radiation is propagated uniformly in all directions with an intensity inversely proportional to the square of the distance. There is also a loss owing to self-absorption. In histological sections the radiation per unit area will increase with thickness up to a certain limit depending on selfabsorption and distance to the film. Rays coming from the same plane which strike the film perpendicularly will have a shorter transit distance than those having an oblique angle of incidence. Consequently, the perpendicular rays will give stronger blackening. Rays from closely spaced cells will partly overlap and influence neighbouring emulsion areas.

The space between the section and the film was estimated at 1 μ and the thickness of the Gevert Dentus Rapid emulsion at about 17 μ (ODEBLAD, 1952). The absorption of the radiation from S^{35} was calculated to be 2 per cent for the rays from the surface of the section. They had to pass an air stratum of at least 1 μ before reaching the emulsion. The rays which originated from the deepest layer of the section had to pass through a cartilage layer of 3 μ (although the embedded sections were 10 μ thick, control measurements revealed, as shown on p. 75, that the thickness of the deparaffined sections had diminished to about 3 μ) and an air stratum of 1 μ and 8 per cent was assumed to be absorbed. These calculations are based on the absorption curve of S^{35} in aluminium, the density difference between cartilage and aluminium being taken into account. It can be assumed that all the S^{35}-containing cells or cell fragments within the selected

rectangle contributed to the blackening of the corresponding area of the contact autoradiograph. The latter assumption can be explained as follows:

Assume that we have two imaginary sections with the same number of cells, say four, per unit volume. In both sections the cells are uniformly distributed throughout the tissue. One section is one cell diameter, the other two cell diameters thick. In the first case the section has four cells under each unit area, in the second case eight cells, although both have the same number of cells per unit volume. If autoradiographs are recorded from these sections the degree of blackening over a unit area of the second section will be almost twice that over a unit area of the first, provided that the cells are equally radioactive and the radiation is sufficiently intense to make the effects of absorption or different transit distances negligible. In other words, provided the section is comparatively thin, the cell content per unit area determines the degree of blackening per unit area of the autoradiograph.

Subjective Estimation of S^{35}-uptake

A semi-quantitative evaluation of the radiosulphur uptake was based on subjective estimations of the intensity of blackening over the various cartilage strata and of the contrast differenes between the cells and the intercellular matrix. When the intracellular and intercellular blackening was the same an attempt was made to establish over which cartilage stratum the blackening was strongest. The various degrees of blackening were classified as follows: Nil (0), weak (+), moderate (++), and strong (+++). This method was used in experiments 1, 2 and 5.

Precautions

The effect of varying section thickness (an important point in experiment 3) was reduced by photometering autoradiographs recorded from several sections (at least 4) from each cartilage. Control measurements of thickness by HALLÉN's method (1955) were made at several points on four patellary sections. Although

the thickness might vary from one point to another in the same section, the average section thickness showed a narrow range of variation and was practically the same for the two sides. The microtome was set to cut 10 μ sections, but after deparaffination they shrunk considerably so that the average thickness of right sections was 2.63 μ (range: 2.14—3.14 μ) and that of left sections 2.77 μ (range: 2.17—3.14 μ).

To ensure uniformity in, partiecularly, experiment 3, all films were exposed simultaneously and developed together. All sections were cut perpendicularly to the joint surface and, autoradiographs having been recorded, stained with haematoxylin-eosin and examined microscopically so that sections with cracks or wrinkles could be rejected.

The S^{35}-uptake of operated cartilage was in experiment 3 only compared with that of unoperated cartilage from the contra lateral joint. Such sources of error as variations in radiosulphate dosage or in the time for the injection were thereby eliminated.

The present experiments were not intended to establish the absolute amount of radiosulphur in a section or per unit volume of the tissue. The degrees of blackness were merely compared in autoradiographs recorded from operated and unoperated cartilage sections and from areas of the same section having different cell densities. Thus, only relative measurements were made. Hence it is justified to use the simplified microphotometric method described here.

CHAPTER X

EXPERIMENTAL

Experiment 1. S^{35} Distribution between Cells and Intercellular Matrix in the Articular Cartilage at Different Times after the Injection

Stripping film autoradiographs were recorded from sections taken from 14 patellary cartilages with operative defects, 24 unoperated patellary cartilages and five normal femorocondylar cartilages obtained from altogether 22 rabbits. The interval between the operation and the S^{35} injection varied from six weeks to 22 months. The periods between the S^{35} injection and the beginning of the fixation were 7, 14, 26, and 48 hours, 7, 17, and 24 days. An S^{35} dosage of 2 mC per kg body weight was given to one animal sacrificed after 7 hours, to two sacrificed after 7 days and to one sacrificed after 24 days.

Experiment 2. S^{35} Distribution in Articular Cartilage Strata

Stripping film autoradiographs were recorded from approximately equal numbers of patellary and femorocondylar cartilage sections from a total of 13 rabbits. 14 sections were from operated and 13 from unoperated knee-joints. The sections were cut outside the defect where all the strata remained uninjured. The same intervals as in experiment 1 occurred between the operation and the S^{35} injection. The periods between the S^{35} injection and the fixation varied from 7 to 56 hours.

Experiment 2 included a study of the S^{35} uptake in degenerated areas of operated articular cartilage, including specimens from 4 patellae covered with plastic film and from 1 joint exposed to the action of formalin solution. Histological sections stained

with haematoxylin-eosin were examined under the microscope and regions with impaired stainability and eradication of cartilage structure, suggesting degeneration, were selected. The intensity of blackness in corresponding regions of the autoradiographs was studied.

Experiment 3. S^{35} Uptake of Operated and Unoperated Articular Cartilage

A. *Autoradiography*

Eleven adult rabbits were used for these experiments. The usual operation of the patellary cartilage was performed unilaterally on each rabbit. The S^{35} injection was made four weeks after the operations.

The animals were killed 48 hours after the injection and mating pieces of patellary and femorocondylar cartilage were faken from each knee. The autoradiographs were exposed tor six days.

B. *Photometry*

The contact autoradiographs from the eleven rabbits were photometered.

C. *Cell Counts in Operated and Unoperated Articular Cartilage*

The cell muclei in the histological sections from the eleven rabbits in experiment 3 A were counted. Operated and unoperated patellary cartilage pairs were compared with respect to the cell density in eight randomly selected regions.

Experiment 4.

A. Correlation between Total Area and Density of Cells and S^{35} Uptake of Operated Articular Cartilage

This series included sections from 25 different regions of altogether 13 pieces of patellary and femorocondylar cartilage from ten knee-joints operated on from 6 weeks to 22 months previously. S^{35} was injected 48 hours before the fixation and

contact autoradiographs were recorded. These were photometered. The degree of blackening was correlated with the cell density and also with the total cell area per unit section area.

B. Correlation between Density of Cells and S^{35} Uptake of Unoperated Articular Cartilage

This series comprised 15 patellary cartilage pieces from eleven unoperated knee-joints. S^{35} was injected 48 hours before the fixation and contact autoradiographs recorded. Each autoradiograph was photometered and the cell density in the corresponding section strips calculated. The cell density per unit area and the degree of blackening were correlated.

Experiment 5. Relationship between S^{35} Uptake and Metachromasia in Articular Cartilage

Contact autoradiographs were recorded from 21 patellary and femorocondylar cartilage sections taken from knee-joints of ten rabbits operated on 6 weeks to 22 months previously. The animals were sacrificed 48 hours, 7 days and 17 days after the S^{35} injection. After toluidine blue staining of the sections, metachromatic areas were related to the blackening of the corresponding autoradiographic recordings.

Control Material

To ascertain whether normal patellary cartilage is capable of blackening Gevaert Dentus Rapid film or stripping film, ten intact patellary cartilage sections were taken from rabbits given no S^{35}. After these sections had been prepared exactly as the radioactive sections, contact and stripping film autoradiographs were recorded. The resulting films were developed and examined under the microscope.

To determine the effect of arthrotomy alone unilateral arthrotomy was performed on two rabbits 17 days and four weeks respectively before receiving S^{35}. Contact autoradiographs were recorded from each patellary and femorocondylar cartilage.

CHAPTER XI

RESULTS

1. S^{35} Distribution between Cells and Intercellular Matrix in the Articular Cartilage at Different Times after the Injection

TABLE 17.

Interval from S^{35} injection to fixation of sections (h = hours; d = days)	No. of rabbits involved	No. of unoperated cartilages (p = patellary; f = femorocondylar)	No. of patellary cartilages with operative defects	S^{35} Distribution
7 h	2	3 p	1 p	Blackening only over chondrocytes in intact cartilages. Slight blackening over chondrocytes and intercellular matrix exhibited by operated cartilage.
14 h	2	3 p	1 p	Blackening only over chondrocytes in all cartilages.
26 h	1	2 p		One cartilage exhibited blackening only over chondrocytes, the other also over intercellular matrix.
48 h	7	1 p	8 p	All cartilages showed severe blackening only over chondrocytes.
7 d	5	7 p	2 p	8 cartilages showed slight overall blackening and the operated 9th blackening only over chondrocytes.
14 d	1	2 p		Both cartilages showed blackening over chondrocytes and intercellular matrix.
17 d	2	2 p 3 f	2 p	4 patellary cartilages showed slight blackening over the chondrocytes and moderate intercellular blackening. 3 femorocondylar cartilages showed blackening over chrondrocytes and intercellular matrix.
24 d	2	4 p 2 f		All the 4 patellary and 2 femorocondylar cartilages showed uniform blackening over chrondrocytes and intercellular matrix.

It appears from Table 17 that articular cartilage has incorporated radiosulphate as soon as 7 hours after the injection.

The S^{35} incorporation in the chondrocytes increased between 7 and 48 hours after the injection (Fig. 23). From 7 to 24 days after the injection the radiosulphate is more evenly distributed between the cells and the intercellular matrix (Figs. 24 and 25), the S^{35} uptake of the latter being most predominant after 24 days. Very rarely the chondrocytes in the autoradiograph appeared white surrounded by a black ring. (Fig. 26). The distribution of the blackening seemed to be much the same in cartilage sections from operated and unoperated joints.

The S^{35} distribution between cells and intercellular matrix was the same for animals given double dosage of S^{35} as for animals given a single dose, that is 1 mC per kg body weight.

2. The S^{35} Distribution in Articular Cartilage Strata

In sections from both operated and unoperated cartilage the ratio of blackening over cells and to that over intercellular matrix seemed to be the same. Table 18 shows the distribution of blackening within the different patellary cartilage strata.

TABLE 18.
Distribution of blackening within patellary cartilage strata.

	No. of sections	Predominance in superficial and intermediate strata	Predominance in radiate stratum or no predominance
Operated	14	8	6
Unoperated	13	2	11

When operated and unoperated joints are assumed to be independent, the probability of obtaining the distribution shown in Table 18 is greater than 0.05 but smaller than 0.1 (p taken from Biometrika Tables). Thus predominance of blackening in the superficial and intermediate strata tended to be more frequent among sections from operated cartilage than among sections from unoperated cartilage. (Figs. 27, 28, 29, 30 and 31).

Microscopical examination of the autoradiographs revealed

that the blackening was reduced over areas characterized by histologically incomplete degeneration and wholly absent over areas presenting complete degeneration (Figs. 32 and 33).

Areas with complete degeneration showed no metachromasia.

3. S^{35} Uptake of Operated and Unoperated Articular Cartilage

Histological examination of the femorocondylar and patellary cartilage sections from operated joints disclosed no signs of either regeneration or degeneration

A. *Autoradiography.*

Microscopical examination of the contact autoradiographs revealed that the degree of blackening was distinctly higher in those recorded from sections of operated patellary cartilage than in those recorded from sections of the contralateral unoperated cartilage. Moreover, autoradiographs recorded from sections of femorocondylar cartilage from the operated side were on the whole darker than the corresponding autoradiographs recorded from the contralateral unoperated cartilage sections (Figs. 34 and 35).

B. *Photometry.*

The photometric results are set out and analysed statistically in Tables 19 to 21.

TABLE 19.

Patellary Cartilages.

Source of variance	df	Sum of squares	s^2	F
Between operated and unoperated	1	18.82	18.82	$F_1 = 171$
Between animals	10	7.24	0.72	$F_2 = 6.54$
Residual	76	8.70	0.11	
Total	87	34.76		

The value of F_1 yields a difference significant on the 0.1 per cent level between the means for operated and unoperated patellary cartilages.

The value of F_2 yields a difference significant on the 0.1 per cent level between the means for the animals.

Mean for operated patellary cartilage = 2.4 R. U. of S^{35}.

Mean for unoperated patellary cartilage = 1.4 R. U. of S^{35}.

TABLE 20.
Femorocondylar cartilages.

Source of variance	df	Sum of squares	s^2	F
Between operated and unoperated	1	8.38	8.38	$F_1 = 59.9$
Between animals	10	18.82	1.88	$F_2 = 13.4$
Residual	76	10.51	0.14	
Total	87	37.71		

The value of F_1 yields a difference significant on the 0.1 per cent level between the means for operated and unoperated femorocondylar vartilages.

The value of F_2 yields a difference significant on the 0.1 per cent level between the means for the animals.

Mean for operated femorocondylar cartilage = 2.4 R. U. of S^{35}.

Mean for unoperated femorocondylar cartilage = 1.8 R. U. of S^{35}.

TABLE 21.
Unoperated patellary versus femorocondylar cartilages.

Source of variance	df	Sum of squares	s^2	F
Between condyles and patellae	1	1.70	1.70	$F_1 = 18.9$
Between animals	10	8.08	0.81	$F_2 = 9,0$
Residual	76	6.72	0.09	
Total	87	16.50		

The value of F_1 yields a difference significant on the 0.1 per cent level between the means for unoperated femorocondylar and patellary cartilages.

The value of F_2 yields a difference significant on the 0.1 per cent level between the means for the animals.

Mean for unoperated femorocondylar cartilage = 1.8 R. U. of S^{35}.

Mean for unoperated patellary cartilage = 1.4 R. U. of S^{35}.

It appears from Table 19 that the S^{35} incorporation in operated patellary cartilage is higher than in unoperated patellary cartilage, the difference being significant on the 0.1 per cent level. An equally significant difference exists between femorocondylar cartilages from operated and unoperated joints (Table 20). Moreover, the unoperated femorocondylar cartilage incorporated more S^{35} than the unoperated patellary cartilage

(Table 21). The latter difference is also significant on the 0.1 per cent level. Nevertheless, the S^{35} uptake of operated patellary cartilage is increased so much that it approaches the S^{35} uptake of femorocondylar cartilage from the operated joints.

C. *Cell Density in Operated and Unoperated Articular Cartilage*

TABLE 22.
Cell Densities.

Source of variance	df	Sum of squares	s^2	F
Between operated and unoperated	1	13.25	13.25	$F_1 = 9.3$
Between animals	10	275.75	27.58	$F_2 = 19.3$
Residual	164	235.03	1.43	
Total	175	524.03		

The value of F_1 yields a difference significant on the 1 per cent level between the means for operated and unoperated cartilages.

The value of F_2 yields a difference significant on the 1 per cent level between the means for the animals.

Mean cell density in operated cartilage = 4.0 cells per unit area ($80 \times 80\ \mu$).

Mean cell density in unoperated cartilage = 4.6 cells per unit area ($80 \times 80\ \mu$).

The average cell density in cartilage from operated joints was 4.0 cells per unit area and in cartilage from unoperated joints 4.6 cells per unit area. Table 22 includes the results and the statistical analysis.

It appears that the cell density in cartilage from operated joints was about 13 per cent lower than in the corresponding cartilage from unoperated joints. This difference is significant. on the 1 per cent level.

This analysis of cell density shows that the increased S^{35} uptake in patellary cartilage from operated joints, as compared with patellary cartilage from unoperated joints, was not dependent on a higher cell density. On the contrary, as the cell density had diminished by about 13 per cent, the chondrocytes existing in operated patellary cartilage must incorporate proportionately more S^{35} than the chondrocytes in unoperated patellary cartilage.

4. A. Correlation between Total Area and Density of Cells and S^{35} Uptake of Operated Articular Cartilage

Table 23 gives (1) the degree of blackening, (2) cell density per unit area and (3) total cell area per unit section area for a number of cartilage sections from operated joints.

TABLE 23.

Correlation between Degree of Autoradiographic Blackening and Density and Total Area of Cells of Operated Patellary Cartilage.

Section No.	Degree of blackening (1)	Number of cells per unit area (2)	Total cell area per unit section area (3)
1	1.7	4.0	5.9
2	1.6	3.0	8.3
3	1.2	2.8	7.3
4	1.4	4.1	6.8
5	1.6	4.6	7.0
6	3.1	5.2	8.3
7	2.5	3.4	10.0
8	2.6	3.4	7.4
9	3.3	5.0	6.8
10	1.8	2.7	8.7
11	2.3	4.2	9.1
12	2.8	5.7	8.4
13	2.3	3.3	7.3
14	3.2	10.0	18.0
15	1.8	3.3	5.0
16	4.0	12.5	16.8
17	3.5	9.3	8.6
18	2.3	9.2	9.5
19	1.2	1.9	4.0
20	1.5	3.9	4.6
21	1.2	2.0	4.1
22	3.5	10.5	8.8
23	1.8	5.3	4.5
24	3.3	7.4	12.4
25	2.1	4.7	7.7

r_{12} = 0.80, 95 % confidence limits: 0.59—0.91.
r_{13} = 0.69, 95 % confidence limits: 0.40—0.85.

As appears from Table 23, the degree of blackening is strongly correlated to the mean cell content of operated cartilage, irrespective of whether the latter be expressed as cell density (number of cells per unit area) or as total cell area per unit section area.

B. Correlation between Density of Cells and S^{35} Uptake of Unoperated Articular Cartilage

TABLE 24

Correlation between Degree of Autoradiographic Blackening and Cell Density of Unoperated Patellary Cartilage.

Section No.	Degree of Blackening	Number of Cells per Unit Area
1	1.4	2.5
2	1.4	3.0
3	1.5	3.0
4	1.1	3.3
5	1.4	3.3
6	1.4	3.5
7	1.6	3.7
8	1.6	4.0
9	1.7	4.1
10	1.7	4.6
11	2.0	5.6
12	1.6	6.5
13	1.9	7.0
14	2.0	8,0
15	2.6	9.6

r = 0.88, 95 % confidence limits: 0.63—0.97.

Table 24 reveals that the S^{35} uptake in unoperated patellary cartilage, as reflected by the degree of autoradiographic blackening, is strongly correlated to the cell density (number of cells per unit area) of such cartilage.

5. Relationship between S^{35} Uptake and Metachromasia in Articular Cartilage

TABLE 25.

Metachromasia / Blackness	+	++	+++
+	1	2	5
++		4	1
+++	2	1	5

All sections of operated femorocondylar and patellary cartilage showed both metachromasia and S^{35} incorporation. As appears from Table 25, the degree of blackening and the intensity of metachromasia did not always coincide (Figs. 36 and 37).

Control Material

None of the autoradiographs recorded from sections taken from rabbits given no S^{35} presented any manifest blackening apart from the usual chemical fog.

Photometric analysis of autoradiographs recorded from patellary and femorocondylar cartilage from two unilaterally arthrotomized rabbits showed lower photometer values on the arthrotomized side than on the contralateral side

CHAPTER XII

DISCUSSION AND CONCLUSIONS

Pioneering the use of S^{35} in metabolic studies, DZIEWIATKOWSKI, BENESCH & BENESCH (1949) demonstrated that articular cartilage possesses a remarkable ability to incorporate radiosulphate. The results of the present investigation bear out that rabbit articular cartilage shows a high affinity for radiosulphur administered in the form of $Na_2S^{35}O_4$. As early as 7 hours after the injection, the autoradiographs began to show S^{35} uptake in the chondrocytes, the amount incorporated increasing between 7 and 48 hours after administration of the radiosulphate. At that time the intercellular matrix also began to manifest radioactivity, and ultimately the blackening over the chondrocytes no longer was dominant compared with that over the intercellular matrix.

Very rarely I came across chondrocytes which in the autoradiograph appeared white and were surrounded by a black ring. ODEBLAD & NORHAGEN (personal communication; submitted for publication 1957) have made similar observations in bronchial and tracheal cartilage both 24 and 48 hours after the radiosulphate injection. Their opinion was that the rings encircled a group of cells functioning an integral unit with respect to S^{35} intake. The fact that in my experiments with rabbit articular cartilage the radioactivity of the chondrocytes as a rule persisted even 24 days after the injection suggests that the turnover of sulphur might be slower in articular cartilage than in bronchial, tracheal or growing epiphyseal cartilage.

Considering that the chondrocytes exhibit the initial signs of S^{35} incorporation in articular cartilage and that completely degenerated cartilage is incapable of taking up S^{35}, the living chondrocytes evidently play an essential part in the sulphur

metabolism of articular cartilage. Using a different approach, BOSTRÖM & MÅNSSON (1953) have also demonstrated that S^{35} incorporation is contingent upon chondrocyte viability. The migration of S^{35} from the chondrocytes to the intercellular matrix may imply that chondroitin sulphate synthesis wholly or partly takes place intracellularly. Thinking along similar lines, BELANGER (1954) stated that the fate of S^{35} as observed at different time intervals after the injection may be interpreted as a visualization of chondroitin sulphate synthesis, secretion, and utilization or turnover. RODÉN (1956) wrote that the S^{35} uptake may be regarded as a direct measure of the total chondroitin sulphate synthesis. Since most inorganic sulphate radiosulphur has left the body after 48 hours (BOSTRÖM, 1952) and little if any is incorporated in thio-amino acids (BOSTRÖM & ÅQUIST, 1952), the high intracellular S^{35} content 48 hours after the injection may be assumed to signify the presence of incompletely or completely synthesized new mucopolysaccharides in the form of chondroitin sulphate inside the chondrocytes.

That the chondrocytes are essential to chondroitin sulphate synthesis is borne out by the present investigation where a strong positive correlation was found between the cell density of articular cartilage and the degree of blackening of the autoradiograph recorded therefrom. The strength of this correlation was about the same whether the cartilage was taken from unoperated or from operated joints, and in the latter case irrespective of whether the degree of blackening was correlated with the number or with the total area of the cells per unit area of the section.

The latter finding leads to quite an interesting speculation. One would expect a large chondrocyte to be capable of incorporating more S^{35} than a small one. Therefore, under standardized conditions and in cartilage from operated joints, the degree of blackness should show a stronger correlation with the total cell area than with the cell density. And, as the chondrocytes tend to be bigger in the radiate stratum than elsewhere in the cartilage, there should be predominance of blackening

over the radiate stratum. Actually the present investigation revealed that the degree of blackening was not more strongly correlated with the total cell area than with the cell density per unit area of the section and also that the blackening tended to predominate over the superficial and intermediate strata rather than over the radiate stratum in operated cartilage. Accordingly all chondrocytes in an operated rabbit articular cartilage would seem to have the same constant capacity for incorporating S^{35} and hence to make equal contributions to the chondroitin sulphate metabolism in such cartilage.

In the present investigation particular attention was paid to the question of how removal from the patellary cartilage of a central chip extending about half way into the radiate stratum affects the incorporation by the remaining articular cartilage of S^{35} injected four weeks after the operation.

The following differences were observed between the operated joint and the contralateral unoperated joint:

1. The S^{35} uptake of operated patellary cartilage was higher,
2. The S^{35} uptake of femorocondylar cartilage articulating with operated patellary cartilage was higher,
3. The S^{35} uptake of the superficial and intermediate strata of operated patellary cartilage tended to be higher.

Ad. 1. Histological examination of the operated patellary cartilage sections revealed that there had not been time for either regenerative or degenerative changes to develop in the four weeks survival period. It is interesting, therefore, that conclusive proof of increased S^{35} uptake was provided by the autoradiographs recorded from these same sections.

Now in addition to a higher than normal S^{35} uptake operated patellary cartilage showed a strong positive correlation between cell density and S^{35} incorporation. Consequently it seems reasonable to suppose that the higher S^{35} uptake of operated than of unoperated patellary cartilage might be due to a higher cell density in the former. However, as shown by the results of experiment 3 C, such was not the case. Cell counts were made in the physically intact parts of operated patellary cartilage

and in the corresponding parts of unoperated patellary cartilage. It appeared that the cell density in operated patellary cartilage far from being higher was significantly lower than in unoperated patellary cartilage, the difference being about 13 per cent. If it is true that all chondrocytes having the same origin and existing under identical conditions possess a constant capacity for incorporating S^{35}, it follows that the operation must have increased the S^{35} capacity of the chondrocytes in the operated patellary cartilage.

One explanation of the augmented S^{35} uptake in operated patellary cartilage could be that the operative intervention — arthrotomy and removal of a superficial cartilage chip — had increased the blood supply to the knee joint and as a consequence stimulated the production of synovial fluid. Yet this explanation must on further consideration be rejected. The radiosulphate injection was given 4 weeks after the operation, at a time when the skin wounds had long since healed and the joint functioned normally without showing any sign of hydrops. Unilateral arthrotomy alone was performed on two rabbits but the S^{35} uptake of both patellary and femorocondylar cartilage was lower in the arthrotomized joint than in the contralateral unoperated joint. Consequently the amount of S^{35} supplied per unit time to the operated knee must have been the same as that reaching the unoperated knee. The most plausible explanation of the greater S^{35} incorporation in patellary cartilage operated upon 4 weeks before the radiosulphate injection than in the contralateral unoperated cartilage is that it reflects a state of stimulated chondrocyte activity in those parts of the patellary cartilage which remained after the operation.

Since the S^{35} uptake may be regarded as a direct measure of total chondroitin sulphate synthesis (Rodén, 1956), the present investigation can be said to have demonstrated that major quantitative changes take place in the chondroitin sulphate metabolism in patellary cartilage following operations thereon. These changes should obviously be interpreted as a regenerative reaction exhibited by the patellary cartilage in response to the operative injury.

Ad. 2. The fact that femorocondylar cartilage articulating with operated patellary cartilage incorporated more S^{35} than the corresponding cartilage from the contralateral unoperated joint is probably just another sign of stimulated chondrocyte activity. The causative factor would then be a mild but uninterrupted traumatization due to articulation against a rough joint surface. It seems, therefore, that articular cartilage which within moderation has to withstand any normal or abnormal strain responds by activation of the chondrocytes and consequently increased total chondroitin sulphate synthesis.

Ad 3. The tendency of the superficial and intermediate strata in operated patellary cartilage to take up more S^{35} than the corresponding regions in the contralateral unoperated joint was observed during the period of predominant intracellular S^{35} incorporation, that is from 7 to 56 hours after the radiosulphate injection. This was clearly visualized in some autoradiographs where, even on the very edge of degenerated areas, the chondrocytes in the intermediate stratum showed a high S^{35} intake (Fig. 33). The augmented S^{35} intake of the superficial and intermediate strata suggests that the chondrocytes therein were highly active. This conforms well with a number of previous observations. Some authors have thus regarded the intermediate stratum as a zone of regeneration providing not only for normal growth (ELLIOTT, 1936) but also for regenerative growth to compensate for the constant attrition of articular cartilage which occurs under normal physiological conditions (SÄÄF, 1950).

It is interesting to consider another finding made in the present investigation, namely that unoperated femorocondylar cartilage incorporated more S^{35} than the ipsilateral unoperated patellary cartilage.

In the present investigation the degree of metachromasia and the degree of autoradiographic blackening did not always coincide. All that definitely can be said is that whenever there was no metachromasia, such as in completely degenerated cartilage blackening was also absent. DUTHIE & BARKER (1955) were unable to relate intensity of metachromatic coloura-

tion to degree of radioactivity in any area of epiphyseal or articular cartilage. BELANGER (1954), on the other hand, obtained good agreement between metachromatic intensity and degree of radioactivity in epiphyseal cartilage. Probably the safest thing to do is to regard the degree of metachromasia as nothing other than a kind of quantitative measure of the existing chondroitin sulphate. On the other hand, the degree of blackening is a measure of the amount of S^{35} incorporated in the articular cartilage during the time elapsed from the radiosulphate injection to the observation. This presupposes that the chondrocytes in the articular cartilage exert some kind of activity and, if so, the S^{35} uptake must reflect a dynamic phase in the chondroitin sulphate synthesis. Consequently, the best and most reliable means of estimating the functional state of or activity in an articular cartilage would seem to be autoradiographic analysis with S^{35} rather than metachromatic estimations.

CHAPTER XIII

PARALLELS BETWEEN THE RESULTS OF PART I AND PART II

Both variants of atypical chondrons showed a high S^{35} uptake. On the other hand, both the chondrocytes and the intercellular matrix in incompletely and completely degenerated cartilage regions possessed respectively little or no ability to incorporate S^{35}. The greater than normal S^{35} incorporation in the atypical chondrons indicates that the sulphate metabolism in their cells was accelerated and was a sure sign of their vitality. It therefore seems fully justified to interprete these new formations as being regenerative.

A markedly increased S^{35} incorporation can, according to BOSTRÖM & MÅNSSON (1953) and others, only occur in cartilage with viable chondrocytes. In this connection it is appropriate to recall the good agreement between histological and autoradiographically visualized degeneration.

It appeared that the atypical chondrons tended to be more numerous in the intermediate stratum and outer radiate stratum than in other strata. In operated patellary cartilages the combined intermediate and superficial strata tended to incorporate relatively higher amounts of S^{35} than other strata. These findings seem to confirm the hypothesis postulated by some authors, for example SILBERBERG (1936) and SÄÄF (1950), that the intermediate stratum has a major part in regenerative growth, at least that growth which compensates for normal attrition. It seems possible that the intermediate stratum also may possess regenerative functions following operative defects on the patellary cartilage. However, the entire intermediate stratum had been cut away at the

site of the defect so that observations in this stratum could be made only outside the margins of the defect.

Examination of sections of articular cartilage from joints operated on four weeks previously disclosed no histologically verifiable regenerative or degenerative changes. However, the cell density in patellary cartilage sections from such joints was about 13 per cent lower than in sections from unoperated patellary cartilage. The present investigation provides no information as to whether this diminution of cell density was absolute or due to a relative increase in the amount of intercellular matrix. Microphotometric analysis of autoradiographs recorded from these cartilage sections had the following results. The S^{35} uptake of operated patellary cartilage was higher than that of normal patellary cartilage and the S^{35} uptake of femorocondylar cartilage articulating against operated patellary cartilage was higher than that of femorocondylar cartilage from unoperated joints. Since the cell density of operated patellary cartilage was subnormal, and each chondrocyte seemed to have a specific capacity for taking up S^{35}, the cells in such cartilage probably incorporated more S^{35} than those in normal patellary cartilage. Autoradiography with S^{35} thus revealed that the operation had given rise to changes in the patellary cartilage, which were manifested as accelerated sulphate metabolism. Yet routine histological procedures failed to disclose any changes suggestive of increased chondrocyte activity in operated patellary cartilage four weeks postoperatively.

It was found that the quantitative S^{35} uptake and the degree of metachromasia was not always parallelled. The S^{35} uptake would seem to provide a better measure of the total chondroitin sulphate synthesis in articular cartilage. As S^{35} incorporation presupposes that an active process takes place in the cells it depicts dynamic activity while metachromasia merely represents a static state.

In conclusion it may be said that autoradiography with S^{35} is a valuable adjunct to routine histological techniques in the study of articular cartilage and in the future it will probably

provide much valuable information about those changes which take place in articular cartilage in response to various injuries. This applies particularly to the study of early osteoarthritic changes, of the healing mechanism and of the final stages of an intraarticular fracture.

CHAPTER XIV

GENERAL SUMMARY

A knowledge of cartilage reactions following operatively created defects is of importance to the surgeon, for it might help him to predict whether and to what extent an operatively injured cartilage is likely to heal.

In the present study the problem of articular cartilage regeneration was narrowed down by introducing a standardized surgical trauma and by exclusively investigating the ability to react of the articular cartilage itself. Hence only shallow defects were made centrally in the patellary cartilage.

The reactions due to operatively created defects in patellary cartilages from 98 adult and 21 yong rabbits were studied by routine histological methods supplemented with metachromatic toluidine blue staining. These studies and their results are described in Part I.

In order to bring into better perspective the findings of Part I and to elucidate the functional conditions in articular cartilage following operative interventions, the sulphate metabolism in the cartilage was investigated with the aid of labelled sulphur. For these experiments autoradiographs were recorded from articular cartilage sections from 33 additional rabbits which had been given intravenous injections of radiosulphate in the form of $Na_2S^{35}O_4$. These experiments and their results are described in Part II.

The results may be formulated thus: —

1. Removal from the patellary cartilage of a central chip projecting about half way into the radiate stratum gave rise to regenerative and degenerative changes, singly or in combination, or failed to produce any manifest changes. Regeneration was

manifested mainly by the development of atypical chondrons which differed from regular chondrons in being composed of an abnormally large number of cells and probably came into existence through proliferation of the chondrocytes themselves. The frequency of atypical chondrons was 3 times higher peripherally than centrally near the actual defect in the patellary cartilage. This dissimilarity may be due to the presumably poorer nutrition of the central parts of the patellary cartilage where the intervention was made. Accordingly the chondrocyte proliferation in the better nourished peripheral parts appears to be a compensatory response. The frequency of atypical chondrons increased with lengthening postoperative survival times. These formations thus occurred in about 33 per cent of all patellary cartilages operated on up to 3 months previously and in approximately 67 per cent of all those operated on 3 to 6 months previously; subsequently the frequency of regeneration showed no significant increase. The atypical chondrons exhibited a high uptake of S^{35}, which presumably should be interpreted as a sure sign of vitality of their component cells. While atypical chondrons occurred in all strata, they tended to predominate in the intermediate and outer radiate strata of the patellary cartilage. Moreover dominance of autoradiographic blackening over the superficial and intermediate strata, as compared with the radiate stratum, tended to be more characteristic of operated than of unoperated patellary cartilage. Taken together these two tendencies suggest that if any of the patellary cartilage strata predominates in attempting to repair cartilage injuries it is the intermediate stratum.

2. Creation of an operative defect in the patellary cartilage also gave rise to degenerative changes. Occurring in all strata, these were twice as common centrally — that is close to the defect — as peripherally in the patellary cartilage. The frequency of degeneration lay practically unchanged at about 45 per cent for all patellary cartilages operated on up to more than a year previously. Degenerated chondrocytes incorporated little or no S^{35}. The histological and autoradiographic visualisations of degeneration were thus in good agreement.

3. No relationship existed between regeneration and degeneration in the patellary cartilage considered as an integral unit. However, when the occurrence of these two modes of reaction was analyzed in the patellary cartilage considered as three layers or zones, it appeared that regeneration would more probably be encountered in a zone lacking degeneration than in a zone with degeneration.

4. Patellary cartilages from young rabbits tended to show a higher frequency of regeneration than those from adults.

5. Application of a plastic film over the defect on the patellary cartilage tended to increase the frequency of degeneration.

6. The chondrocytes in both operated and unoperated articular cartilage showed S^{35} incorporation commencing 7 hours and increasing up to 48 hours after the radiosulphate injection. After that time the intercellular matrix also began to show radioactivity and after 7 days the radioactivity of the chondrocytes equalled that of the intercellular matrix.

7. Statistical analysis was applied to the results of an experimental series where the animals were sacrificed 48 hours after receiving radiosulphate and 4 weeks after undergoing unilateral operation on the patella. It appeared that the operated patellary cartilage incorporated more S^{35} than the corresponding cartilages on the contralateral unoperated side. The augmented S^{35} uptake was regarded as evidence of increased cell activity and as a first sign of a regenerative or reparative reaction shown by the articular cartilage in response to the injury. The femorocondylar cartilage articulating with the operated patella also incorporated more S^{35} than the corresponding cartilage from the contralateral unoperated joint. Apart from a reduction by about 13 per cent of the cell density, no signs of regeneration or degeneration could be manifested histologically 4 weeks after the operation.

8. The cell density in both operated and unoperated articular cartilage was strongly correlated to the degree of autoradiographic blackening, that is to the S^{35} uptake. In operated cartilage this correlation was almost equally strong whether

the degree of blackening was correlated to the total cell area per unit area of the section or to the number of cells per unit area of the section. Irrespective of their size the chondrocytes in a given articular cartilage would seem to possess the same unit capacity for taking up S^{35}, this unit capacity being higher in operated than in unoperated cartilage.

9. The degree of metachromasia in histological sections was not consistently parallelled by the degree of blackening of the corresponding autoradiograph. Conceivable reasons for this inconsistency are discussed.

REFERENCES

ASBOE-HANSEN, G., ed.: Connective Tissue in Health and Disease. Munksgaard, Copenhagen 1954.

AXHAUSEN, G.: Arch. klin. Chir. *99*: 519, 1912.

— — Arch. klin. Chir. *124*: 543, 1923.

BANFIELD, W. G.: In Connective Tissue in Health and Disease. Ed. by G. Asboe-Hansen. P. 151. Munksgaard, Copenhagen 1954.

BAUER, W., ROPES, M. W. & WAINE, H.: Physiol. Rev. *20*: 272, 1940.

BÉLANGER, L. F.: Canad. J. Biochem. & Physiol. *32*: 161, 1954.

BENNETT, G. A. & BAUER, W.: J. Bone & Joint Surg. *17*: 141, 1935.

BENNETT, G. A., BAUER, W. & MADDOCK, S. J.: Am. J. Path. *8*: 499, 1932.

BENNINGHOFF, A.: Lehrbuch der Anatomie des Menschen. Bd I. Urban & Schwarzenberg, München & Berlin 1949.

— — Ztschr. Zellforsch. *2*: 783, 1925.

BOSTRÖM, H.: J. Biol. Chem. *196*: 477, 1952.

— — Arkiv för kemi. *6*: 43, 1953.

BOSTRÖM, H. & GARDELL, S.: Acta chem. scandinav. *7*: 216, 1953.

BOSTRÖM, H. & JORPES, E.: Experientia *10*: 392, 1954.

BOSTRÖM, H., JORPES, E., MÅNSSON, B., RODÉN, L. & WESTERMARK, A.: Arkiv för kemi. *8*: 469, 1955.

BOSTRÖM, H. & MÅNSSON, B.: Arkiv för kemi. *6*: 23, 1953.

BOSTRÖM, H., ODEBLAD, E. & FRIBERG, U.: Acta path. et microbiol. scandinav. *32*: 516, 1953.

BOSTRÖM, H., RODÉN, L. & WESTERMARK, A.: Nature *176*: 601, 1955.

BOSTRÖM, H. & ÅQVIST, S.: Acta chem. scandinav. *6*: 1557, 1952.

BOURGHARDT, S., BRATTGÅRD, S. O., HYDÉN, H., JIEWERTZ, B. & LARSSON, S.: J. Scient. Instruments *30*: 464, 1953.

BRATTGÅRD, S. O., HALLEN, O. & HYDÉN, H.: Biochim. Biophys. Acta *10*: 486, 1953.

BRODIN, H.: Acta orthop. scandinav. Suppl. 20, 1955.

BURCKHARDT, H.: Arch. klin. Chir. *132*: 706, 1924.

CIOCIOLA, F.: Policlinico (sez. chir.) *28*: 299, 1921.

CLARK, E. R. & CLARK, E. L.: Am. J. Anat. *70*: 167, 1942.

DONIACH, I. & PELC, S. R.: Brit. J. Radiol. *23*: 184, 1950.

DUTHIE, R. B. & BARKER, A. N.: J. Bone & Joint Surg. *37 B*: 304, 1955.

DZIEWIATKOWSKI, D. D.: J. Biol. Chem. *178*: 197, 1949.

— — I. Biol. Chem. *178*: 389 1949.

— — J. Biol. Chem. *189*: 187, 1951.

Dziewiatkowski, D. D., Benesch, R. E. & Benesch, R.: J. Biol. Chem. *178*: 931, 1949.
Ekholm, R.: Acta anat. Suppl. 15, 1951.
— — Acta anat. *24*, 329, 1955.
Ekholm, R. & Norbäck, B.: Acta orthop. scandinav. *21*: 81, 1951.
Elliott, H. C.: Am. J. Anat. *58*: 127, 1936.
Fasoli, G.: Arch. per le sc. med. *29*: 365, 1905.
Fisher, A. G. T.: Brit. J. Surg. *10*: 52, 1922—1923.
— — Brit. M. J. *II*: 390, 1939.
Floderus, S.: Acta path. et microbiol. scandinav. Suppl. 53, 1944.
Gersh, I. & Catchpole, H. R.: Am. J. Anat. *85*: 457, 1949.
Gies, T.: Deutsche Ztschr. Chir. *18*: 8, 1882.
Gross, J., Bogoroch, R., Nadler, N. J. & Leblond, C. P.: Am. J. Roentgenol. *65*: 420, 1951.
Gross, J. & Schmitt, F. O.: J. Exper. Med. *88*: 555, 1948.
Gurlt, E. F.: Beiträge zur vergleichenden pathologischen Anatomie der Gelenkkrankheiten. Berlin 1853.
Gussenbauer, C.: Arch. klin. Chir. *12*: 791, 1871.
Hallén, O.: Acta anat. Suppl. 25, 1955.
Ham, A. W.: Histology. 2. ed. Lippincott, Philadelphia, London & Montreal 1953.
Hammar, J. A. H.: Bidrag till ledgångarnes histologi. Diss. Upsala 1892.
— — Arch. mikroskop. Anat. *43*: 266, 1894.
Hansen, Fr. C. C.: Undersögelser over Bindevævsgruppen. Den hyaline Bruskgrundsubstans. Diss. Köbenhavn 1900.
— — Ztschr. wissenschaftl. Mikr. *25*: 145, 1908.
Hildebrand, O.: Arch. klin. Chir. *115*: 443, 1921.
Hintzsche, E.: Ztschr. mikr.-anat. Forsch. *25*: 320, 1931.
Hirsch, C.: Acta chir. scandinav. Suppl. 83, 1944.
Hirsch, C., Paulson, S., Sylvén, B. & Snellman, O.: Acta orthop. scandinav. *22*: 175, 1952.
Holmdahl, D. E. & Ingelmark, B. E.: Acta anat. *6*: 309, 1948.
Hultén, O. & Gellerstedt, N.: Acta chir. scandinav. *84*: 1, 1940/41.
Hultkrantz, J. W.: Das Ellenbogengelenk und seine Mechanik. Diss. Stockholm 1897.
Hunter, W.: Phil. Tr. Roy. Soc., London *9*: 267, 1743.
Häbler, C.: Beitr. klin. Chir. *134*: 602, 1925.
Häggqvist, G.: Acta chir. scandinav. *65*: 180, 1929.
Ingelmark, B. E. & Ekholm, R.: Acta Soc. med. upsalien. *53*: 61, 1948.
Ingelmark, B. E. & Sääf, J.: Acta orthop. scandinav. 17,303, 1948.
Ishido, B.: Virchows Arch. path. Anat. *244*: 424, 1923.
Ito, L. K.: Brit. J. Surg. *12*: 31, 1924/25.
Key, J. A.: J. Bone & Joint Surg. *13*: 725, 1931.
— — J. Bone & Joint Surg. *15*: 67, 1933 A.

KEY, J. A.: M. J. & Rec. *138*: 369, 1933 B.

KING, E. S. J.: J. Path. & Bact. *41*: 117, 1935.

KLING, D. H.: Arch. Surg. *23*: 543, 1931.

KROMPECHER, S.: Die Knochenbildung. Fischer, Jena 1937.

KROMPECHER, S. & GOERTTIER, K.: Verhandl. anat. Gesellsch. *46*: 43, 1938.

KULONEN, E.: In Connective Tissul in Health and Disease. Ed. by G. Asboe-Hansen. P. 70. Munksgaard, Copenhagen 1954.

KÖLLIKER, A.: Verhandl. physikal.-med. Gesellsch. in Würzburg *3*: 1, 1852.

LAYTON, L. L.: Proc. Soc. Exper. Biol. & Med. *76*: 596, 1951.

LEIDY, J.: Am. J. M. Sc. *17*: 277, 1849.

LISON, L.: Arch. biol. *46*: 599, 1935.

LLOYD-Roberts, G. C.: J. Bone & Joint Surg. 37 B: 1, 1955.

LUBOSCH, W.: Bau und Entstehung der Wirbeltiergelenke, Jena 1910.

LUTWAK-MANN, C.: Biochem. J. *34*: 517, 1940.

MANCINI, R. E.: Anat. Rec. *101*: 149, 1948.

MARKOWITZ, J.: Experimental Surgery. 3. ed. William & Wilkins, Baltimore 1954.

MAXIMOW, A. & BLOOM, W.: Textbook of Histology. 5. ed. Saunders, Philadelphia & London 1948.

MATTHEWS, B. F.: Brit. Med. J. ii: 660, 1953.

MC CLUNG, J. R.: Mc Clung's Handbook of Microscopical Technique for Workers in Animal and Plant Tissues. Ed. by R. Mc Clung Jones. 3. ed. Harper, New York 1950.

MEYER, K.: In Connective Tissue in Health and Disease. Ed. by G. Asboe-Hansen. P. 54. Munksgaard, Copenhagen 1954.

MEYER, K., PALMER, J. W. & SMYTH, E. M.: J. Biol. Chem. 119: 501, 1937.

MONTAGNA, W.: Anat. Rec. *103*: 77, 1949.

MÜLLER, W.: Biologie der Gelenke. Barth, Leipzig 1929.

ODEBLAD, E.: Acta radiol. Suppl. 93, 1952.

— — Acta radiol. *45*: 323, 1956.

ODEBLAD, E. & BOSTRÖM, H.: Acta path. et microbiol. scandinav. *31*: 339, 1952.

— — Nord. med. *48*: 962, 1952.

ODEBLAD, E. & NORHAGEN, Å.: Submitted for publication 1957.

OGSTON, A.: J. Anat. & Physiol. *10*: 49, 1875/76.

PAGET, J.: Lectures on Surgical Pathology. Vol. 1, 263. Longman, London 1853.

PAYR, E.: München. med. Wchnschr. *70*: 1333, 1923.

PEARSON, E. S. & HARTLEY, H. O. eds.: Biometrika Tables for Statisticians. Vol. 1. Cambridge University Press, 1954.

PELC, S. R.: Nature *160*: 749, 1947.

PENNISI, A.: Policlinico (sez. chir). *11*: 489, 1904.

PERSSON, B. H.: Acta Soc. med. upsalien. Suppl. 2, 1953.

Policard, A.: Physiologie générale des articulations à l'état normal et pathologique. Masson, Paris 1936.

Prudden, T. M.: Am. J. M. Sc. *82*: 360, 1881.

Redfern, P.: Monthly. J. Med. Sc. *13*: 201, 1851.

Rimann, H.: Virchows Arch. path. Anat. *180*: 446, 1905.

Rodén, L.: On the Biosynthesis of Sulpho-Mucopolycaccharides. Diss. Stockholm. Uppsala 1956.

Romeis, E.: Mikroskopische Technik. 15. verb. Aufl. Leibnitz, München 1948.

Rosenthal, O., Bowie, M. A. & Wagoner, G.: J. Cell. & Comp. Physiol. *17*: 221, 1941.

Rössle, R.: Wachstum und Altern. Bergmann, München 1923.

Sacks, S.: South African M. J. *23*: 475, 1949.

Schaffer, J.: Das Knorpelgewebe. In Handbuch der mikroskopischen Anatomie des Menschen. Hrsg. von W. v. Möllendorff. Bd II: 2, 210. Springer, Berlin 1930.

Schiller, S., Mathews, M. B., Goldfaber, L., Ludowieg, J. & Dorfman, A.: J. Biol. Chem. *212*: 531, 1955.

Schiller, S., Mathews, M. B., Jefferson, H., Ludowieg, J. & Dorfman, A.: J. Biol. Chem. *211*: 717, 1954.

Schmaus, H., Herxheimer, G.: Grundriss d. Pathol. Anat. 10. Aufl. Bergmann, Wiesbaden 1912.

Schmidt, A.: Beitr. klin. Chir. *130*: 142, 1924.

Schmieden, V.: Arch. klin. Chir. *62*: 542, 1900.

Seggel, R.: Deutsche Ztschr. Chir. *75*: 453, 1904.

Shands, A. R., Jr.: Arch. Surg. *22*: 137, 1931.

Sheehan, J. F.: J. Morphol. *82*: 151, 1948.

Silberberg, M.: Proc. Soc. Exper. Biol. & Med. *34*: 333, 1936.

Sylvén, B.: J. Bone & Joint Surg. *29*: 745, 1947.

— — Acta orthop. scandinav. *18*: 21, 1948.

— — J. Bone & Joint Surg. *30 A*: 158, 1948.

Sylvén, B. & Malmgren, H.: Lab. Invest. *1*: 413, 1952.

Sylvén, B., Paulson, S., Hirsch, C. & Snellman, O.: J. Bone & Joint Surg. *33 A*: 333, 1951.

Sääf, J.: Acta orthop. scandinav. Suppl. 7, 1950.

Tizzoni, G.: Arch. per le sc. med. 2: 28, 1878.

Weichselbaum, A.: Virchows Arch. path. Anat. *55*: 217, 1872.

Wells, H. G.: Chemical Pathology. Saunders, Philadelphia 1920.

Virchow, R.: Die krankhaften Geschwülste. Bd 1, 460. Hirschwald, Berlin 1863.

Wislocki, G. B., Bunting, H. & Dempsey, E. W.: Am. J. Anat. *81*: 1, 1947.

PLATES

PART I

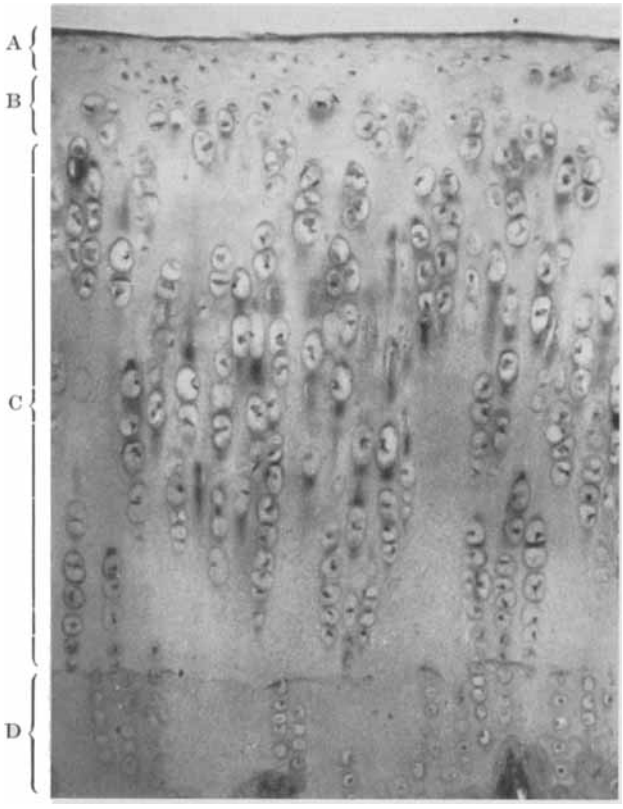

Fig. 1. Normal patellary cartilage from adult rabbit. Haematoxylineosin-staining, × 200. A = Superficial Stratum. B = Intermediate Stratum. C = Radiate Stratum. D = Calcified Cartilage Stratum.

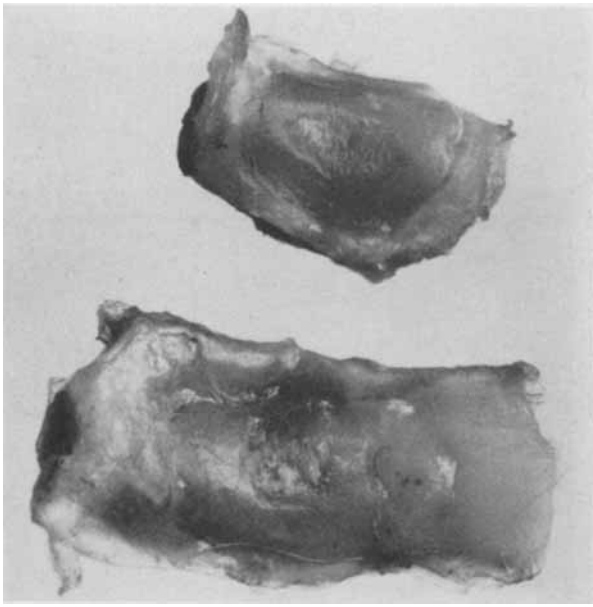

Fig. 2. Photomacrograph of patellae from young rabbit subjected to bilateral patellary operation 14 months previously. The operative defect remains visible. Approx. × 5.

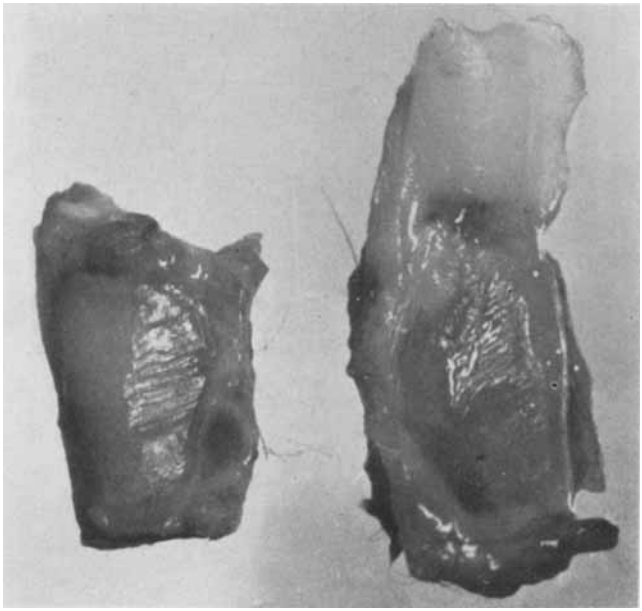

Fig. 3. Photomacrograph of patellae from adult rabbit subjected to bilateral patellary operation 12 months previously. The operative defect remains visible. Approx. × 5.

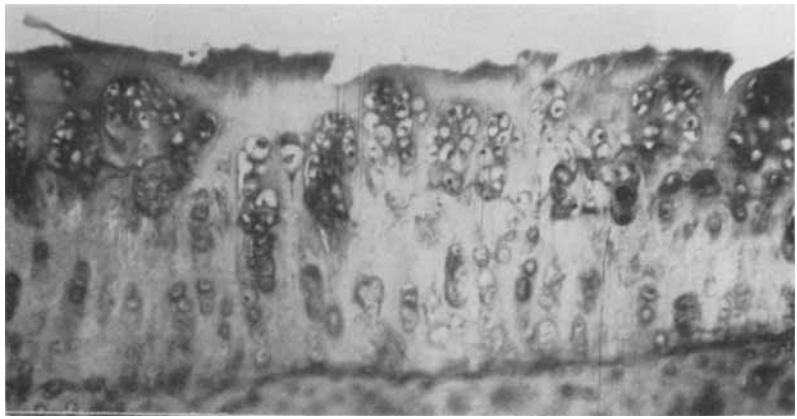

Fig. 4. Patella from young rabbit operated on 14 months previously. The intermediate stratum contains numerous atypical chondrons of variant A surrounded by cartilage of fairly normal appearance. Haematoxylin-eosin staining, × 140. A = Atypical Chondrons of Variant A.

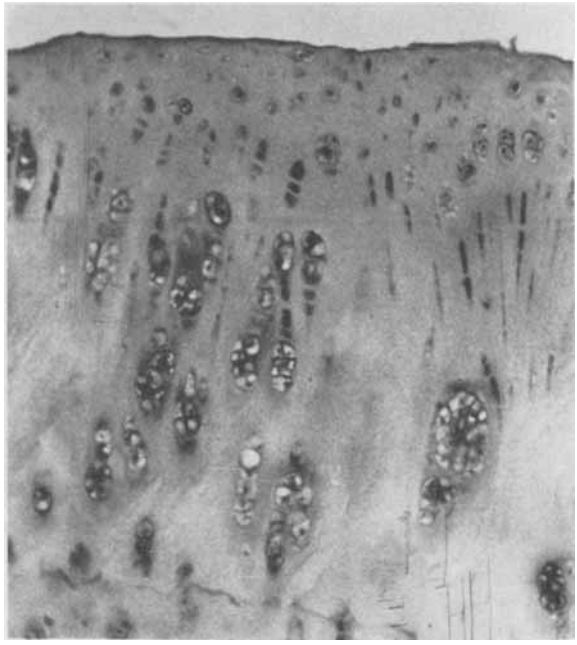

Fig. 5. Patella from young rabbit operated on 8 months previously. The intermediate stratum contains atypical variant A chondrons and the deeper cartilage layers are degenerated. Haematoxylin-eosin staining, × 140. A = = Atypical Chondrons of Variant A.

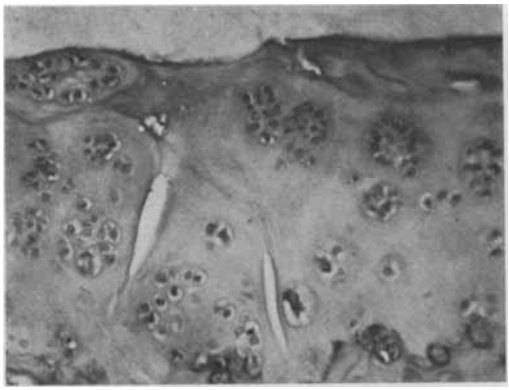

Fig. 6. Patella from adult rabbit operated on 5 months previously. Atypical chondrons of variant A may be seen fairly uniformly distributed through the various strata. Haematoxylin-eosin staining, × 220.

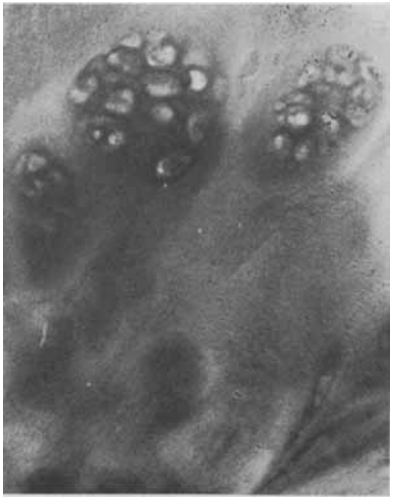

Fig. 7. Patella from adult rabbit operated on 6½ months previously. Atypical variant A chondrons having metachromatic intercellular matrix are present. Toluidine blue staining, × 450.

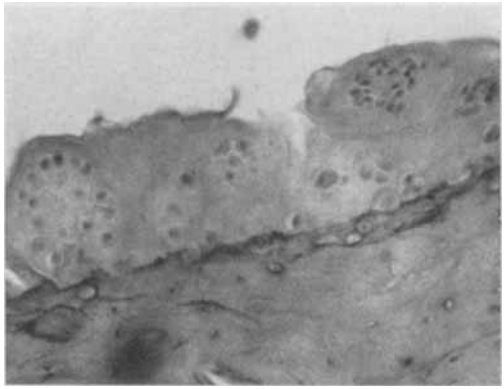

Fig. 8. Patella from adult rabbit operated on 6.5 months previously. Atypical variant A chondrons are evident in the deepest radiate stratum. Haematoxylin-eosin staining, × 210.

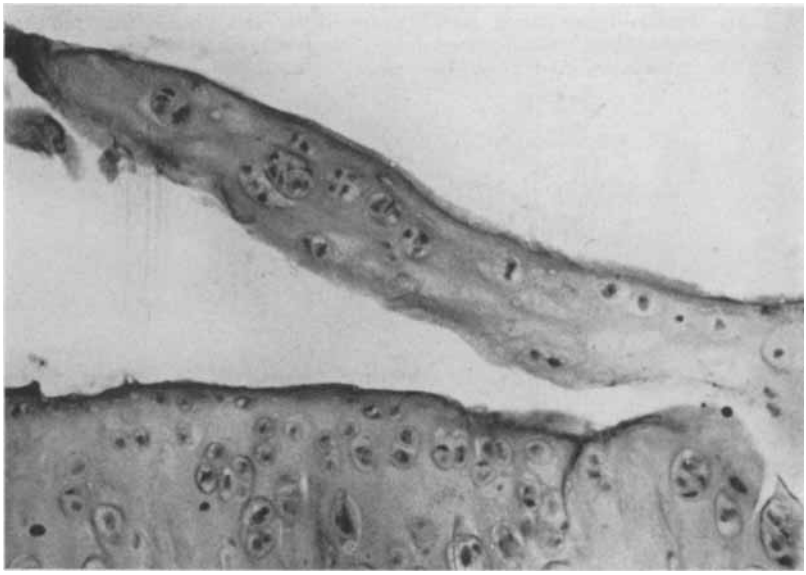

Fig. 9. Patella from young rabbit subjected to partial detachment of cartilage shaving 6 months previously. The shaving contains small atypical variant A chondrons near the free end. Haematoxylin-eosin staining, × 210.

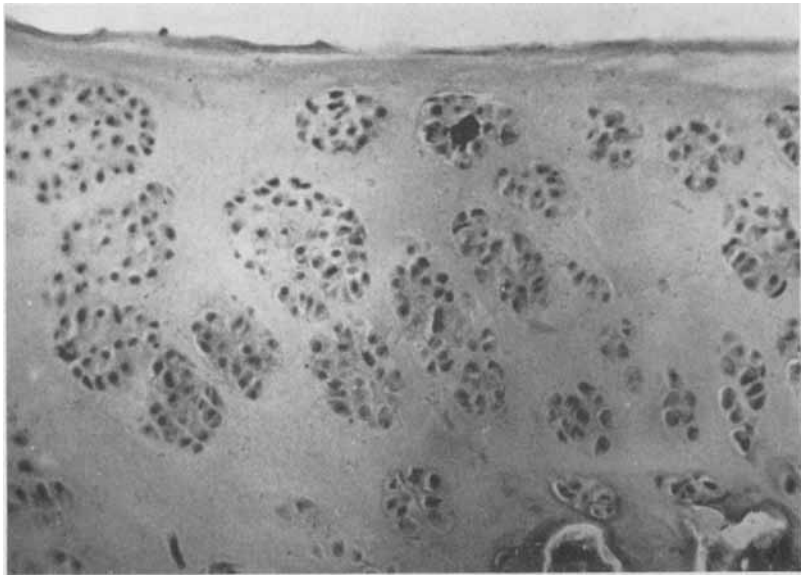

Fig. 10. Patella from young rabbit operated on and plastic film applied over the defect about 2 months previously. Numerous, large atypical chondrons of variant B are visible. Haematoxylin-eosin staining, × 210.

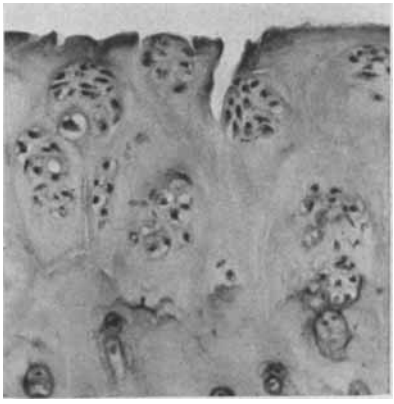

Fig. 11. Patella from adult rabbit operated on 6 months previously. Atypical variant B chondrons are seen situated in incompletely degenerated cartilage. Haematoxylin-eosin staining, × 200.

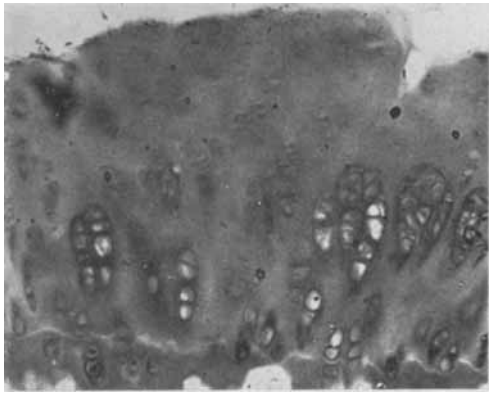

Fig. 12. Patella from young rabbit, the same as in figure 10. Atypical variant B chondrons having strongly metachromatic capsules are present. Toluidine blue staining, × 175.

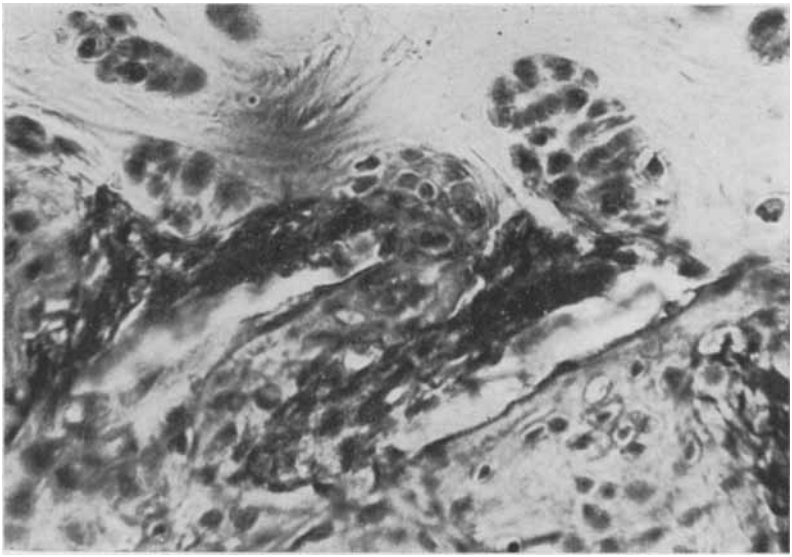

Fig. 13. Patella from adult rabbit operated on 2.5 months previously. Atypical variant B chondrons have apparently grown up through a preformed bone marrow canal. Haematoxylin-eosin staining, × 420.

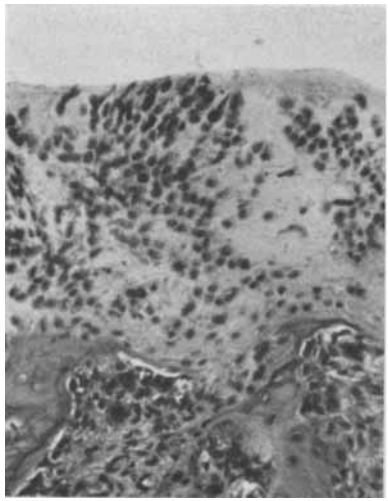

Fig. 14.

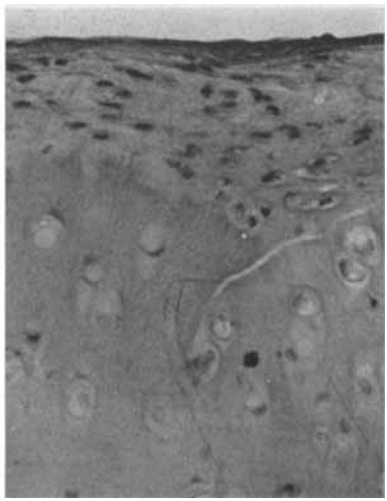

Fig. 15.

Fig. 14. Patella from adult rabbit operated on 10 weeks previously. Cell swarms are present. Haematoxylin-eosin staining, × 180.

Fig. 15. Patella from adult rabbit operated on 4 months previously. Cell islands composed of fibrocytoid units are manifest on the surface of the defect. Haematoxylin-eosin staining, × 290.

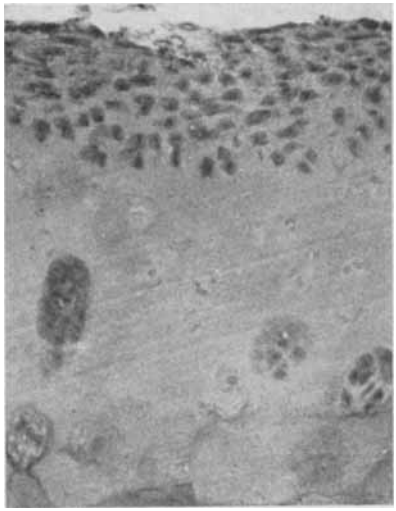

Fig. 16. Patella from young rabbit operated on 6 weeks previously. Both atypical chondrons surrounded by degenerated cartilage and superficial cell islands are visible. Haematoxylin-eosin staining, × 290.

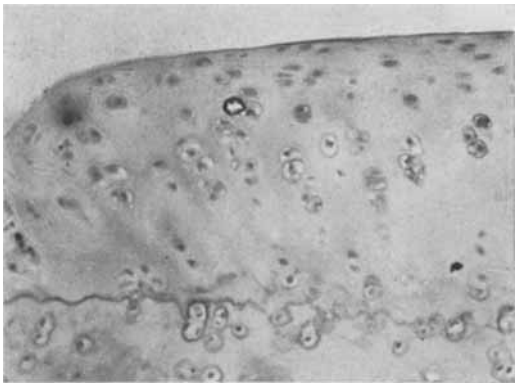

Fig. 17. Patella from young rabbit operated on one year previously. A restored superficial stratum partially covers the defective area. Haematoxylin-eosin staining, × 160.

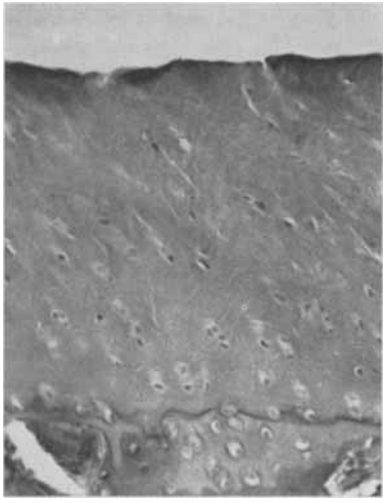

Fig. 18. Patella from young rabbit operated on and plastic film applied over the defect 3 months previously. Complete degeneration is present. Haematoxylin-eosin staining, × 200.

Fig. 19. Patella from adult rabbit operated on 2.5 months previously. The cartilage exhibits severe degeneration approaching necrosis. Haematoxylin-eosin staining, × 130.

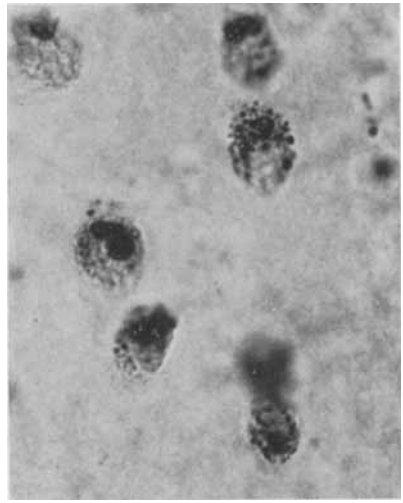

Fig. 20. Frozen section of normal patella stained with Sudan III. Fat globules are present in all chondrocytes, × 860.

PART II

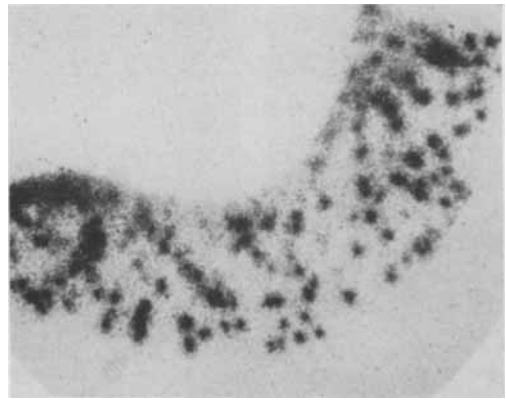

Fig. 23. Autoradiograph recorded from section of patellary cartilage operated on 5 months previously and obtained from rabbit given S^{35} 48 hours previously. Blackening appears only over cells and chondrons, × 40.

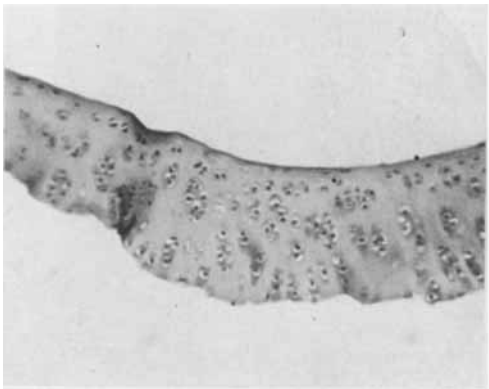

Fig. 24 A.

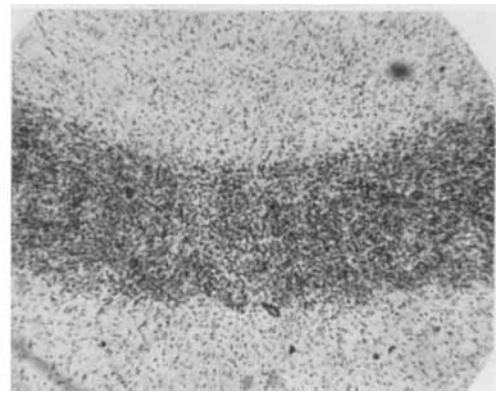

Fig. 24 B.

Fig. 24 A. Section of unoperated patellary cartilage, haematoxylin-eosin staining, × 40.

Fig. 24 B. Autoradiograph recorded from section in Fig. 24 A. The blackening is equally intense over chondrocytes and intercellular matrix. The animal had been given S^{35} 14 days previously, × 40.

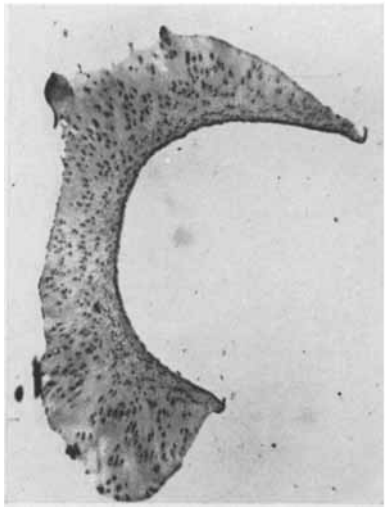

Fig. 25 A.

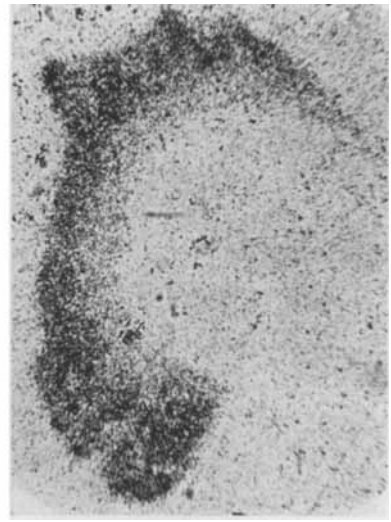

Fig. 25 B.

Fig. 25 A. Femorocondylar cartilage section from unoperated joint, haematoxylin-eosin staining, × 40.

Fig. 25 B. Autoradiograph recorded from section in Fig. 25 A. The animal had been given S^{35} 24 days previously. The blackening is equally intense over chondrocytes and intercellular matrix, × 40.

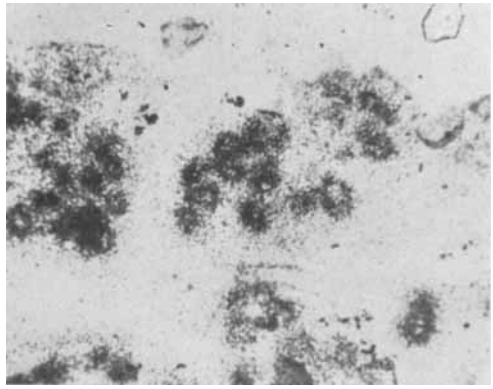

Fig. 26. Stripping film autoradiograph recorded from femorocondylar cartilage section from joint operated on 3.5 months previously. The animal had received S^{35} 48 hours previously. The blackening takes the form of rings encircling individual chondrocytes or clusters of chondrocytes, × 200.

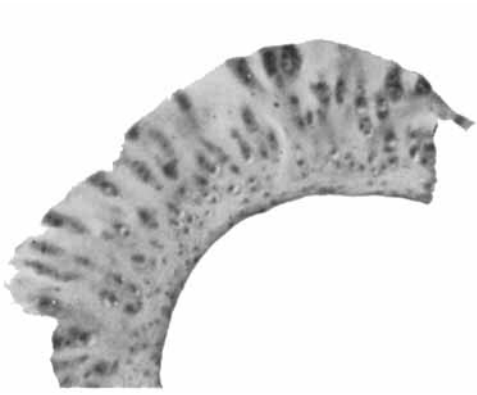

Fig. 27 A.

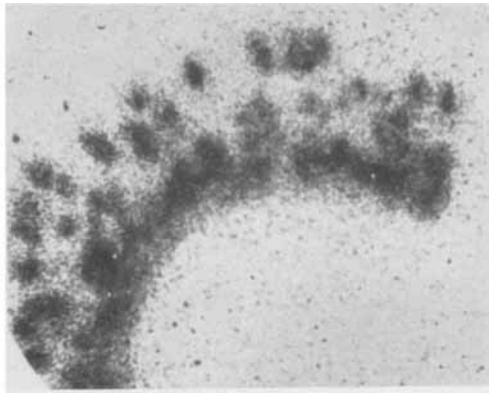

Fig. 27 B.

Fig. 27. A. Patellary cartilage section from operated joint, haematoxylin-eosin staining, × 80.

Fig. 27 B. Autoradiograph recorded from section in Fig. 27 A. The animal had been given S^{35} 48 hours previously. The blackening predominates over the superficial and intermediate strata, × 80.

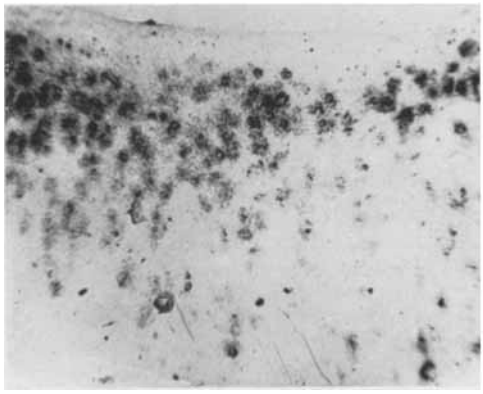

Fig. 28.

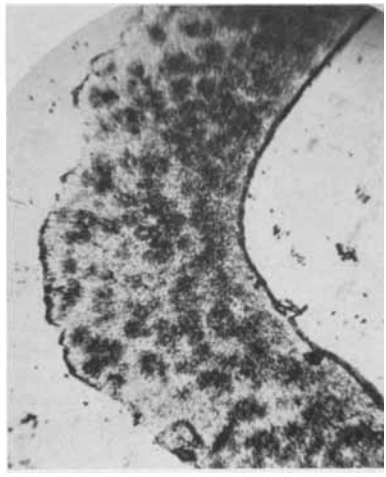

Fig. 29.

Fig. 28. Stripping film autoradiograph recorded from femorocondylar cartilage section from operated joint. The animal had received S^{35} 48 hours previously. The blackening predominates over the cells in the intermediate stratum, × 160.

Fig. 29. Stripping film autoradiograph recorded from femorocondylar cartilage section from joint operated on 7 weeks previously. The animal had received S^{35} 7 days previously. The blackening is present over cells and intercellular matrix, and seems to predominate over the intermediate stratum, × 85.

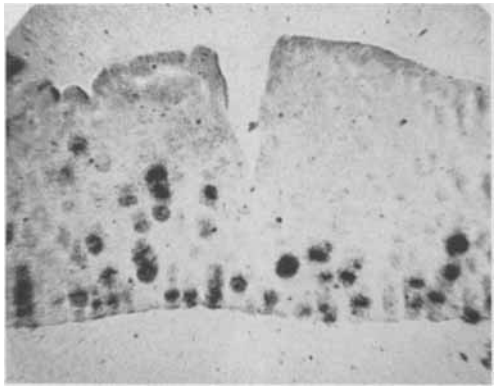

Fig. 30. Stripping film autoradiograph recorded from section of patellary cartilage operated on 22 months previously. The animal had received S^{35} 48 hours previously. Blackening is absent near the surface where the cartilage is degenerated and present over the cells in the radiate stratum, × 90.

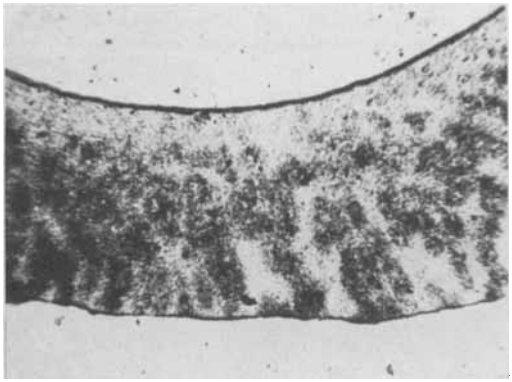

Fig. 31. Stripping film autoradiograph recorded from unoperated patellary cartilage section from animal given S^{35} 7 days previously. Predominating over the radiate stratum, the blackening is present over both cells and intercellular matrix, × 80.

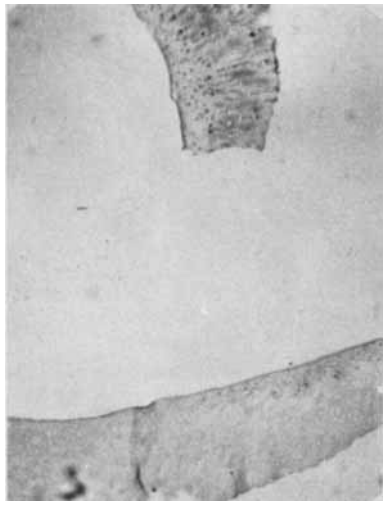

Fig. 32 A. Section of patellary cartilage operated on 22 months previously. In the part near the bone any persisting cell structures are indistinct and there is complete degeneration. Haematoxylin-eosin staining, × 40.

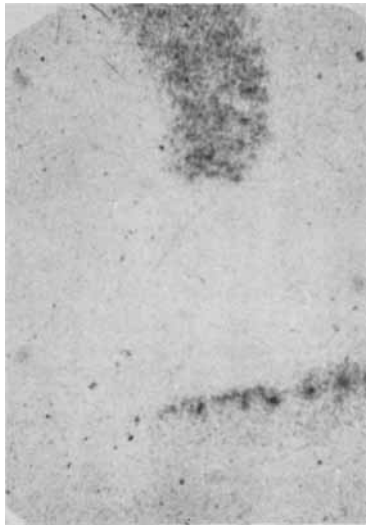

Fig. 32 B. Autoradiograph recorded from section in Fig. 31 A. The animal had received S^{35} 48 hours previously. The blackening is slight over the cell remnants and absent over the completely degenerated areas, × 40.

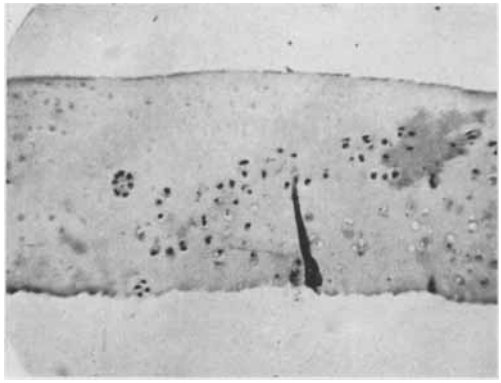

Fig. 33 A. Section of femorocondylar cartilage from joint operated on 4 months previously. The left portion presents ghost cells and diminished stainability, the right shows unimpaired nuclear stainability particularly in the intermediate stratum. Haematoxylin-eosin staining, × 80.

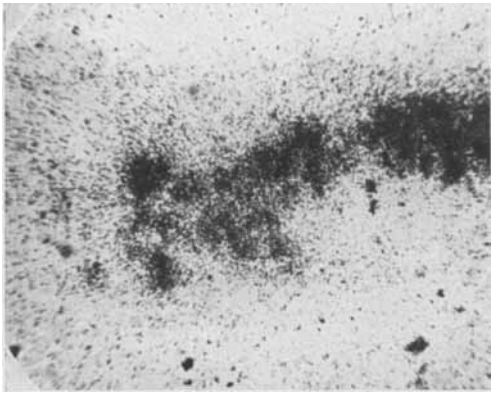

Fig. 33 B. Autoradiograph recorded from section in Fig. 32 A. The animal had received S^{35} 48 hours previously. There is blackening over cells with persisting nuclear stainability especially over an atypical chondron but little or none over degenerated areas, × 80.

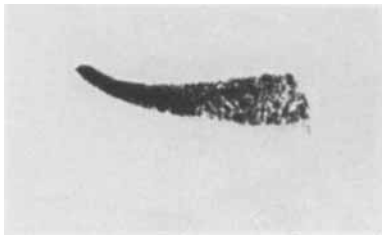

Fig. 34 A.

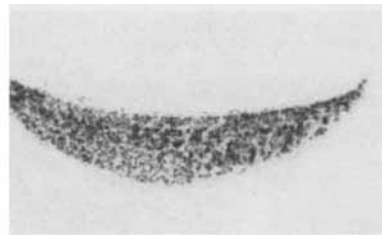

Fig. 34 B.

Fig. 34 A. Autoradiograph recorded from section of operated patellary cartilage. The animal had received S^{35} 48 hours previously. The degree of blackening is very high, × 20.

Fig. 34 B. Autoradiograph recorded from section of unoperated patellary cartilage. The same animal as in fig. 34 A. The degree of blackening is lower than in Fig. 34 A, × 20.

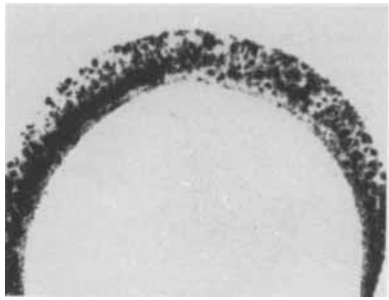

Fig. 35 A.

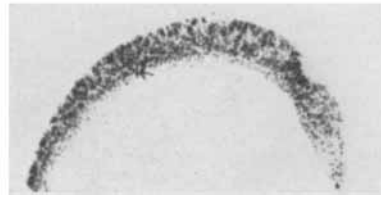

Fig. 35 B.

Fig. 35 A. Autoradiograph recorded from femorocondylar cartilage section from operated joint. The animal had recived S^{35} 48 hours previously. The degree of blackening is very high, × 20.

Fig. 35 B. Autoradiograph recorded from femorocondylar cartilage section from unoperated joint. The same animal as in fig. 35 A. The degree of blackening is lower than in Fig. 35 A, × 20.

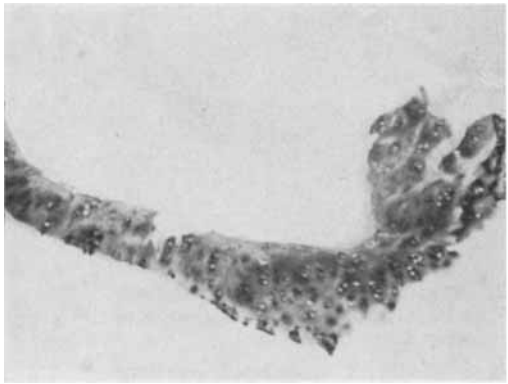

Fig. 36 A.

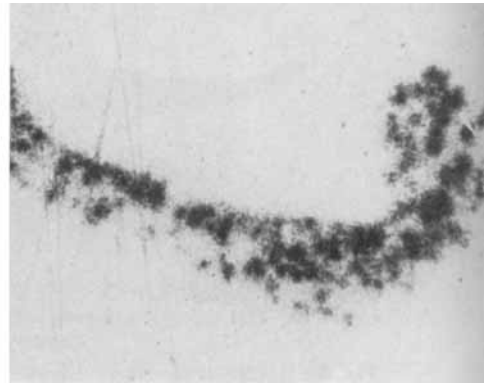

Fig. 36 B.

Fig. 36 A. Section of femorocondylar cartilage from joint operated on 8 months previously. The entire section is characterized by marked metachromasia. Toluidine blue staining, × 40.

Fig. 36 B. Autoradiograph recorded from section in Fig. 35 A. The animal had received S^{35} 48 hours previously. The degree of blackening is high over the entire section, × 40.

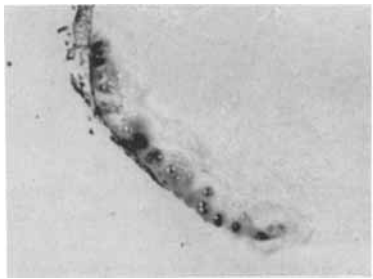

Fig. 37 A.

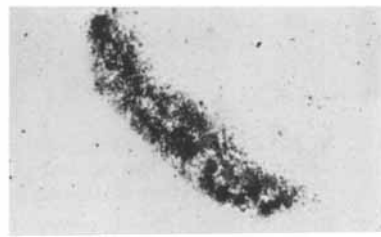

Fig. 37 B.

Fig. 37 A. Section of patellary cartilage operated on 8 months previously. The superficial portion presents slight the other portions strong metachromasia. Toluidine blue staining, × 40.

Fig. 37 B. Autoradiograph recorded from section in Fig. 36 A. The animal had received S^{35} 48 hours previously. The degree of blackening is high over the entire section including those parts with diminished metachromasia, × 40.