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EXPERIMENTAL OSTEOARTHRITIS

A Survey

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The question of how osteoarthritis arises has not yet been answered. However, experiments in animals can help to solve this problem. With advancing age cartilaginous non-symptomatic changes become more and more common in every joint in the human body, reaching close to 100 per cent (Collins 1949). This is not osteoarthritis. Trueta (1968) stated that clinical osteoarthritis arises first when the underlying bone reacts. Osteoarthritis is a disease not only of the cartilage but also of the joint capsule and the bone.

Cartilage is a tissue without blood vessels, lymph vessels and nerves. Thus in adults cartilage is an isolated tissue without supervision or control by blood cells and macrophages and without a pain response with superficial wounds. Immunoglobulins can with difficulty pass through healthy cartilage. In osteoarthritic cartilage, the occurrence of immunoglobulins IgA and IgG has been demonstrated recently (Cooke et al. 1980).

The diffusion of nutrients in growing cartilage occurs both from the bone and from the synovium; in mature cartilage it occurs only from the synovium. The mineralized layer and the tidemark constitute an absolute obstacle for molecules, even the smallest ones, i.e. hydrogen (Ogata et al. 1978). The speed of passage of nutrients from the joint into the basal layer is determined by the molecular size of the solute, the electrical charge, the thickness of the cartilage and the degree of motion in the joint (Maroudas 1973). The proteoglycans have a negative charge, the size of which depends on the amount of glycosaminoglycans. Therefore, small non-ionic solutes such as urea and glucose distribute themselves in principle equally between the extracellular water in the matrix and the joint fluid. Small cationic solutes are attracted and the small anionic solutes are partially excluded.

The avascularity of the cartilage can be explained by the existence of a special factor which can be extracted by guanidine hydrochloride according to Eisenstein et al. (1973) and Langer et al. (1976). This substance inhibits the growth of vessels *in vivo* and vascular endothelial cells in culture. This factor is not the same as the trypsin inhibitor factor which can also be isolated from cartilage (Sorgente & Dorey 1980). Normal cartilage cannot be penetrated by vessels; conversely, mineralized cartilage is easily penetrated by vessels. When the cartilage becomes degenerated in osteoarthritis it is probable that the antivascular factor disappears and the cartilage therefore becomes penetrated by vessels and destroyed.

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Experimental osteoarthritis

It is easy to produce joint changes in experimental animals. Practically every long-standing injury to the cartilage, either mechanical or chemical, results in degenerative cartilage changes. These changes can also be produced by certain disturbances of hormone regulation.

The following mechanical procedures have been used:
Immobilization
Compression of the joint surfaces
Resection of parts of joints including meniscectomy
Making the joint unstable
Producing incongruence of the hip joint

The changes occurring after the different procedures are mainly of the same type: loss of metachromasia, clustering of the chondrocytes, fibrillation, clefts, blistering, osteophytes, loss of cartilage with denudation of subchondral bone, ingrowth of vessels. Biochemically, loss of sulphated glycosaminoglycans has been demonstrated but there is no loss of collagen. At the same time there is an increase in the turnover of sulphate and even collagen.

Immobilization. The changes in the different tissues of the joint after immobilization are described by Evans et al. (1960), Hall (1963) and Langenskiöld et al. (1979). The changes in soft tissue after immobilization studied by Videman et al. (1979) are, for example, a thickening of the knee joint capsule and the synovial membrane as well as an increased uptake of ^{35}S-sulphate in these structures, in the menisci and in the medial collateral ligament. The changes are reversible up to a certain period of immobilization and then they become irreversible (Videman et al. 1979).

Compression of cartilage (Salter & Field 1960, Trias 1961, Ginsberg et al. 1969, Thompson & Bassett 1970) with springs or with other means results in necrosis of areas of the cartilage and around these areas there occur degenerative changes, loss of metachromasia, clustering, fibrillation etc.

Resection of parts of joints e.g. resection of the patella (Bruce & Walmsley 1942, Cohn 1944), or one of the condyles of the knee joint (Thompson & Bassett 1970). Due to the loss of contact the opposite joint surfaces undergo regressive changes. The cartilage, therefore, takes on the appearance of fibrous tissue with fibrocyte-like cells. The cartilage becomes vascularized, the mineralized layer disappears, and the subchondral bone decreases in thickness. Resection of a meniscus also leads to degenerative changes according to Moscowitz et al. (1973).

Instability of knee joints has been brought about either by section of the anterior cruciate ligaments in dogs (Marshall & Olsson 1971, McDevitt & Muir 1976) or by cutting several structures in rabbits: the medial collateral ligament, both cruciate ligaments and removal of the medial meniscus (Hulth et al. 1970, Telhag & Lindberg 1972). Both procedures give progressive degenerative changes of the joint which are similar to human osteoarthritis.

The first-mentioned model on dogs has been thoroughly investigated biochemically by McDevitt & Muir (1976) and McDevitt et al. (1977). The purified proteoglycans extracted from the cartilage differed from those of normal cartilage tissue in that the quotient chondroitin sulphate, keratin sulphate was different. The proteoglycans were also more easily extracted. An increased hydration and swelling in non-fibrillated regions occurred, this also being typical of human osteoarthritis. They found vascular proliferation in the synovial membrane which became more intense with time. An increased thickness of the synovial membrane with yellowish discolouration and the presence of villous folds and adhesions was also noted.

The corresponding changes in the rabbit model are similar but probably much more profound. With time, the cartilage becomes more and more destroyed with cartilage degeneration: cell clusters, fibrillation, ulceration, clefts, osteophytes.

Ehrlich et al. (1975) have used this model for biochemical studies of the cartilage. They found a decrease in proteoglycans, an increase in acid phosphatase, and increases in the rate of synthesis of protein and glycosaminoglycans. There

was no change in collagen content and the ^{3}H-thymidine incorporation was not significantly increased.

Incongruence of the hip joint in dogs has been produced by Inerot et al. (1980) through an extraarticular osteotomy of the innominate bone (reverse). There occurred progressive macroscopic degeneration and the size of the proteoglycan monomer decreased.

The subchondral bone reacts to greater loads e.g. during compression experiments, by bone formation and the subchondral bone becomes heavier. At loss of contact, e.g. on resection of a part of a joint or immobilization, the subchondral bone becomes rarefied and thinner. Bohr (1976) found increased labelling of the subchondral bone with fluor and technetiumpyrophosphate and tetracycline in arthropathy due to instability of the knee joint according to the method of Telhag (1972). Bach-Christensen (1980) found the same but mainly in the osteophytes, the increased uptake in the subchondral bone itself coming much later. McDevitt & Muir (1976) found thickening of the synovial membrane and villous folds. Videman et al. (1979) also found changes of the synovial membrane after immobilization.

Experimental studies on the subchondral bone and the synovial membrane are still relatively rare compared with the number of studies on human osteoarthritis. These investigations have shown hypermetabolism of the subchondral bone with higher contents of alkaline and acid phosphatases (Reimann et al. 1977), and increased vascularity in the synovial membrane.

Mitosis in adult joint cartilage

Chondrocytes in adult cartilage do not divide. Whenever the cartilage has sustained an injury, cell clusters occur. Mitotic figures have been found in articular cartilage when the joint was compressed (Crelin & Southwick 1960, Trias 1961). Hulth et al. (1970), who first described the rabbit model of the unstable knee, demonstrated thymidine labelled chondrocytes as early as the fifth day and up to 5 months after the operation. Labelled chondrocytes could also be found in human osteoarthritis of the hip after *in vitro* labelling (Hulth et al. 1972). De Palma et al. (1966) have shown that chondrocytes around an artificial defect in joint cartilage are capable of taking up radioactive thymidine. Havdrup et al. (1975) found after scarification of the rabbit patella that the labelled cells were found not only in the injured patella but also scattered in the joint, in the tibial and femoral condyle. Labelled chondrocytes also occurred in the contralateral joint which had been arthrotomized only or had been left intact. This is contrary to the results obtained in experimental osteoarthritis, where the contralateral joint did not show any labelled chondrocytes.

The number of labelled chondrocytes after scarification of the patella or femoral groove was most numerous at 14 days after the operation. After that time they decreased in number and gradually disappeared (Havdrup & Telhag 1978). It is probable that the labelling of cells at a distance from the scarification is due to a tissue specific substance released by the incision in the cartilage and spread through the joint and to other joints via the blood. This factor can either be a stimulating factor or a factor which removes molecules bound to the cell membrane which normally prevent mitosis from appearing (so-called chalones). No labelled cells were found in rabbits which were only arthrotomized.

Proteolytic enzymes also result in labelled cells in the mature cartilage. Papain exerts its action on the protein GAG binding sites but has no effect on collagen fibres. Crude or inactive papain administered intravenously produces vast alterations resulting in depletion of the polysaccharides of the matrix. Intravenous injection of papain results in mitosis spread in the different joint cartilages (Havdrup et al. 1981). Intraarticular administration of purified papain results in changes similar to human osteoarthritis as described by Murray (1964) and Bentley (1971). Both papain and trypsin injected intraarticularly result in labelled cells. Havdrup & Telhag (1977) and Havdrup (1979) have found that smaller doses of papain and of trypsin result in labelled cells without considerable degenerative changes of the cartilage. It is probable that both enzymes act mitogenically by removing mitosis inhibiting

molecules situated on the cell membranes. The same explanation can be valid for those mitoses that appear in human or experimental osteoarthritis. It has been established that lysosomal enzymes become effective with the development of osteoarthritis (see Ali 1964 or Chrisman 1969).

Injuries of the cartilage must be divided into those which concern only the cartilage and those which also engage the subchondral bone. Shaving or scarification of the superficial cartilage does not result in healing of the defects, as shown by e.g. Meachim (1963), even when studied for up to 66 months, as shown by de Palma et al. (1966). Injuries penetrating to the subchondral bone result in the development of cartilage filling out the defect. Healing of cartilage, therefore, seems to be impossible without access to cells coming from bone marrow. It is impossible to know exactly which cells are the origin of the new chondrocytes. Cheung et al. (1980) showed that during the healing of surgically induced deep defects in mature rabbits, hyaline cartilage comes from the subchondral bone underneath the lesion. At the end of the first month mushroom-shaped chondroid buds were seen sprouting from the subchondral bone and marrow. By the 10th week these buds fused into a cartilaginous plug filling the lesion. They could also show that the repaired cartilage synthesized type II collagen, the same as in normal hyaline cartilage. In the long run, after a year or so, the repaired cartilage changes its character and becomes more scar-like, as also shown by Hjertquist & Lemperg (1971). The repair of cartilage does not take its origin from the damaged cartilage itself but like an embryonal process it is a form of induction of undifferentiated cells (Owen 1980). Salter et al. (1975) have shown in rabbits that full-thickness defects in articular cartilage heal through the formation of new hyaline cartilage with continuous passive motion of the joint, but with fibrous tissue in the case of immobilization.

The other problem which is interesting to speculate about is why superficial cartilage wounds do not heal. Zucker-Franklin & Rosenberg (1977) have put forward a theory. The role collagen has in platelet aggregation is possibly crucial in connective tissue repair. Rutherford & Ross (1976) and Kaplan et al. (1979) have shown that thrombocytes are essential for the growth of fibroblasts and arterial smooth muscle cells in culture. The aggregated platelets form a firm substrate for the deposition of fibrin as well as for proliferation of fibroblasts. Zucker-Franklin & Rosenberg (1977) found that cartilage collagen can also aggregate thrombocytes but first after removing the proteoglycans by trypsin. They could also show that the treatment of rabbit knee joints with intraarticular trypsin 1 week before the injection of blood resulted in adhesion and aggregation of platelets on the surface of the lesions. This implies that scarification in cartilage cannot heal because its collagen is covered by proteoglycans plus it lacks access to thrombocytes from the blood.

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