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TRYPSIN-INDUCED MITOSIS IN THE ARTICULAR CARTILAGE OF ADULT RABBITS

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Full-grown rabbits were injected in the knee joints with solutions of trypsin of various concentrations. The animals were sacrificed 2 weeks after the trypsin injection. Twenty-four hours before sacrifice they received 40 μCi ^{3}H-thymidine intra-articularly. The changes in the knee joints were then studied by histological and autoradiographical methods. The injection of trypsin did not result in the development of osteoarthritis. However, autoradiography revealed that the chondrocytes started to divide after the injection. The mitosis of the chondrocytes can thus not be due to degeneration of the cartilage. The explanation put forward is that the mitosis of the chondrocytes may be the result of a decrease in the concentration of a growth controlling factor (chalone) initiated by the administration of trypsin.

Key words: trypsin; cartilage; mitosis; autoradiography; chalones

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Reversible collapse of rabbit ears after intravenous administration of papain is due to the release of chondroitin sulphate from the protein-polysaccharide complex of the matrix (Thomas 1956, Spicer & Bryant 1957, Bryant et al. 1958, Tsaltas 1958, McElligott & Potter 1960). Intravenous or intra-articular administration of papain results in degenerative and necrotic changes, somewhat similar to those seen in human osteoarthritis (Murray 1964, Bentley 1971, Farkas et al. 1974, 1976, Havdrup & Telhag 1977). It has never been possible to demonstrate with certainty mitotic figures in normal adult joint cartilage (Crelin & Southwick 1960, 1964, Mankin 1963, 1964, 1968, Hulth et al. 1970, Telhag 1972). Mitosis of the chondrocytes is induced in adult joint cartilage in association with the development of osteoarthritic changes (Crelin & Southwick 1960, 1964, Hulth et al. 1970, Telhag 1972). When papain is administered intra-articularly, the degenerative changes are accompanied by the appearance of mitosis of the chondrocytes (Havdrup & Telhag 1977). Papain solutions of decreasing concentrations result in progressively less degeneration of the cartilage, whereas the occurrence of mitosis remains high (Havdrup & Telhag, to be published).

In the present study trypsin, another proteolytic enzyme, is studied. Trypsin, injected intravenously in rabbits, does not produce the same changes as seen after papain administration (Blumberg & Ogston 1957). Trypsin splits the peptide bonds which follow one or other of the two positively charged side-chains, i.e. lysine and arginine.

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The aim of the present investigation was to find out if trypsin, injected intra-articularly, induces degenerative changes in adult joint cartilage and if it initiates mitosis of the chondrocytes.

MATERIAL AND METHODS

The material consisted of 10 full-grown rabbits with roentgenographically closed epiphyseal lines. The rabbits were caged under normal conditions and given a normal diet. The animals weighed 3600–5550 g (mean 4075 g). Solutions of trypsin (Trypure®, Novo) in 0.9 per cent NaCl were prepared in the following concentrations: 3750 E/ml, 7500 E/ml, 15000 E/ml, 30000 E/ml and 60000 E/ml. Each concentration was used in two rabbits. They were injected in the right knee joints with 0.2 ml of the trypsin solution. In all the animals, the left knee joints were used as controls, and were injected with 0.2 ml of 0.9 per cent NaCl. The animals were killed 2 weeks after the injection of trypsin by an intravenous injection of Nembutal® (Abbot). Twenty-four hours before sacrifice, 40 μCi of ^{3}H-thymidine was injected into both knee joints. No signs of infection were seen.

The knee joints were dissected free and fixed in 10 per cent formalin solution. The patella, tibia and femur were dissected free and treated separately. The tibia and femur were divided in the frontal plane into two halves with a circular saw. The pieces were decalcified in 50 per cent formic acid and 20 per cent sodium citrate. Afterwards, the pieces were embedded in paraffin and cut into sections 5–7 μ thick. The sections were stained with haematoxylin-eosin, according to van Gieson, safranin-O and toluidine. Autoradiograms of routine histological sections were prepared from both knee joints according to the dipping method with Ilford K2 liquid emulsion. The autoradiograms were exposed for 3 weeks after which they were developed in Gevaert X-ray developer G 230 and fixed in Gevaert X-ray fixer G 305. The sections were then stained through the emulsion with Mayer's haematoxylin. As a rule 10–12 autoradiograms from each knee joint were examined.

RESULTS

The animals all survived the experimental period and none showed signs of infection of the knee joints. There were no signs of a generalized toxic reaction to the administered trypsin.

Histological examination

None of the left control knee joints showed signs of degeneration. The staining properties of the matrix were normal.

After the injection of trypsin in the right knee joints, only sparse histological changes were seen.

3750 E/ml: No changes in the histology could be seen. Staining properties were normal.

7500 E/ml: The cartilage seemed to be normal except for suspected fibrillation in one of the joints. Slight proliferative changes in synovial and osteoblastic cells were seen at the margins. The staining properties of the matrix were normal.

15000 E/ml: One of the knee joints showed signs of degeneration with necrosis of chondrocytes, fibrillation and cluster formation. At the joint margins there was proliferation of synovial cells and proliferation of osteoblasts. The cartilage of the other knee joint was normal. Slight proliferation of synovial cells could be seen at the joint margins. Osteoblastic activity was low. The staining properties of the matrix were normal in both knee joints.

30000 E/ml: The structure of the cartilage was normal, but some of the chondrocytes appeared to be swollen in comparison with normal chondrocytes and others showed signs of necrosis with fragmentation of the nucleus. The proliferation of cells at the margins was still more pronounced than mentioned above. Osteoblastic activity was seen at the joint margins. The staining properties of the matrix were normal.

60000 E/ml: The cartilage showed no histological signs of degeneration. One of the knee joints showed the presence of a marginal osteophyte. Proliferation of synovial cells and osteoblastic activity were seen at the joint margins.

Autoradiographical evaluation

None of the control knee joints showed signs of ^{3}H-thymidine labelling.

In the knee joints injected with trypsin, labelled chondrocytes were seen in all parts of the joints, i.e. in the patella, femur and tibia. Seven of the ten knee joints studied showed ^{3}H-thymidine labelled cells. The knee joints showing no labelling belonged to the animals receiving dilutions containing 3750 E/ml, 30000 E/ml and 60000 E/ml. The labelled chondrocytes were distributed in the following manner:

	Patella	Tibia	Femur	Total	Number of autoradiograms	Labelled cells/section
3750 E/ml	0	3	5	8	19	0.4
7500 E/ml	6	16	4	26	23	1.1
15000 E/ml	0	5	20	25	25	1.0
30000 E/ml	0	1	0	1	22	0.04
60000 E/ml	1	6	4	11	21	0.5

DISCUSSION

In order to study the metabolism and the treatment of osteoarthritis, it is very useful to have experimental models resulting in joint changes resembling those seen in human osteoarthritis.

Degenerative joint disease can be induced in many different ways, i.e., mechanical, chemical, infectious, immunological and endocrinological (Gardner 1960). Many of these methods, however, result in changes resembling those seen in rheumatoid arthritis.

By making a joint unstable (Hulth et al. 1970, Telhag 1972), it has been possible to produce degenerative and reparative joint changes similar to those seen in osteoarthritis. When degenerative changes appear in the cartilage, the chondrocytes recover their ability to divide (Telhag 1972). Scarification of the articular cartilage of the patella in adult rabbits does not result in generalized degenerative changes of the cartilage. The scarification, however, gives rise to scattered mitosis of the chondrocytes in all parts of the joint cartilage (Havdrup et al. 1975).

After intra-articular injection of concentrated papain solutions, extensive degenerative and necrotic changes of the joint cartilage appear. In the remaining cartilage, single dividing chondrocytes can be seen (Havdrup & Telhag 1977). When weaker solutions of papain are injected, the degenerative and necrotic changes are not so prominent but, in spite of this, the number of dividing chondrocytes is higher.

In the present investigation another proteolytic enzyme, trypsin, was used. Only slight degenerative changes were found histologically in the articular cartilage. Autoradiographically, mitosis of the chondrocytes was found after injection of trypsin in all the above-mentioned dilutions, and the greatest number of dividing cells were seen when the solution containing 15000 E/ml was used.

When articular cartilage is damaged, the accompanying mitosis of the chondrocytes might be explained as an attempt to restore cartilage. It is more difficult to explain why mitosis occurs when the degenerative changes are sparse or even absent. If the theories of chalones are applied to this experiment, the findings can be explained. Dormant tissue cultures have been stimulated to new growth by the addition of trypsin (Simms & Stillman 1937). This was suggested to be due to the elimination of an inhibitor to cell growth which had been produced by the cells themselves and was present in the culture medium. A chalone has been defined as "an internal secretion produced by a tissue for the purpose of controlling by inhibition the mitotic activity of the cells of the same tissue". The existence of chalones in epidermis has been proved by Bullough & Laurence (1960), and later in other tissues by other workers (Saetren 1956, Mohr et al. 1968, Rytömaa & Kiviniemi 1969, Lasalvia et al. 1970, Houck & Hennings 1973, Maharajan & Batra 1976 and Janakivedi et al. 1976). When applied to joint cartilage, the chalone might be reduced in the joint cavity either by chemical (papain and trypsin) or mechanical (osteoarthritis and scarification) factors.

In earlier investigations it has been mentioned that chalone preparations are destroyed by the addition of trypsin solutions

(Boldingh & Laurence 1968, Lasalvia et al. 1970, Houck et al. 1973).

The present results do not, at least, contradict the theory of chalones applied to articular cartilage. The histological findings are slight and the changes in one of the animals, belonging to the animals having received 15000 E/ml, can hardly be explained as due to the injection of trypsin. The animals which have received more concentrated solutions of trypsin do not show corresponding changes. Possibly the relatively grave changes were present even before the injection of trypsin. One has to consider that the animals are old, and that "normally" occurring osteoarthritis can be present. The appearance of dividing chondrocytes can be regarded as an effect of chalone reduction due to trypsin digestion. Why peak mitosis occurs with a trypsin concentration of 15000 E/ml is difficult to explain. One explanation is that in the groups of animals receiving the 30000 and the 60000 E/ml trypsin solutions, respectively, only one of the animals in each group showed mitosis of cartilage. The number of labelled cells/section must then be diminished.

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