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LOCALIZED REGRESSIVE ARTICULAR CARTILAGE CHANGES IN THE HIP JOINT OF THE RABBIT FOLLOWING AN INDUCED SYNOVITIS

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A talcum induced synovitis in the hip joint of the rabbit, which is known to cause articular cartilage hyperplasia followed by femoral head protrusion and joint incongruency, has in the present experiment also been shown to lead to localized regressive articular cartilage changes. The articular cartilage of the hip joints in 40 rabbits was examined histologically, at intervals, following induction of such a talcum synovitis. Regressive changes in the form of loss of surface chondrocytes and glycosaminoglycans sometimes accompanied by fibrillation, were found in the area of the femoral head articular cartilage which had become flattened following the head protrusion. Chondrocyte cloning facilitated subsequent cartilage repair. The biomechanical disturbance in the joint following the induced synovitis is felt to have caused the regressive changes. The experiment is considered to have some significance in connection with Legg-Calvé-Perthes' Syndrome (L.C.P.S.) in children.

Key words: cartilage degeneration; Perthes' disease; synovitis

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In a previous study (Gershuni-Gordon & Axer 1974) we have shown that a synovitis induced in the rabbit's hip joint by the intra-articular injection of a surgical talc suspension stimulated thickening of articular cartilage which decreased the acetabular capacity vis-a-vis the increased head size. Thus the femoral head protruded from the joint as demonstrated radiologically, and due to abnormal pressure effects the femoral head developed a flattening superiorly. It was decided to investigate further these induced effects on the joint cartilage by histological examination using special stains so as to relate the biochemical changes to changing morphology, the final objective being to advance the understanding of the aetiology of Legg-Calvé-Perthes' Syndrome (L.C.P.S.) in children.

MATERIAL AND METHODS

Forty immature female rabbits, 21 to 28 days old, were anaesthetized with open ether and after exposing the right hip joint capsule an intra-articular injection of surgical talc (magnesium tetrasilicate) suspension was performed; the left hip served as the control (Gershuni-Gordon & Axer 1974). The animals were allowed to ambulate normally. The hip joints were X-rayed at 2 weeks postoperatively and just prior to killing. The radiological Medial Joint Space (M.J.S.) was measured from the medial edge of the capital epiphysis to the acetabular "tear-drop" to check whether the joint cartilage thickened in response to the induced synovitis and had caused joint incongruency (Gershuni-Gordon & Axer 1974).

The majority of animals were killed in groups at weekly intervals from 2 to 6 weeks, since the previous study had shown this period to be the most significant for witnessing the effects of an

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induced synovitis; however, a smaller number of animals were followed from 7 to 11 weeks. The hip joint capsules were carefully opened and the articular surfaces, after rinsing with physiological saline solution, were painted with Indian ink and rinsed again with saline (Meachim 1972); any areas retaining the ink particles were recorded. To obviate the discrepancies known to occur in the histochemical techniques used in staining cartilage matrix and especially glycosaminoglycans, the specimens from the experimental and control hips were treated in an exactly similar fashion from fixation to staining. Thus the hip joints were fixed in formalin and then decalcified in a formic acid buffer solution. The femora were sectioned in the coronal plane and the acetabula in the plane passing vertically through the centre of the acetabular notch. After paraffin wax embedding of complementary right and left hemi-sections in the same block, sections at 7 μ were cut on the microtome. Simultaneously right and left sections were stained on the same slide with haematoxylin and eosin or safranin 0 and fast green. Safranin 0 selectively stains glycosaminoglycans to an intensity of colour which is proportional to the content of the glycosaminoglycans in the cartilage (Rosenberg 1971).

The sections were examined grossly and microscopically, always comparing the right operated side with the left control side. Measurement was made of the following features:

1. Articular cartilage thickening
2. Femoral head cartilage flattening
3. Loss of glycosaminoglycans as evidenced by decreased safranin 0 and increased fast green counter-staining
4. Loss or death of chondrocytes
5. Cloning of chondrocytes
6. Articular cartilage fibrillation

RESULTS

Radiographic Medial Joint Space (M.J.S.)

Enlargement of the M.J.S. was previously shown to correlate with thickening of the joint cartilage (Gershuni-Gordon & Axer 1974). Thirty-five out of 40 animals had a significant enlargement of the M.J.S., i.e., 0.3 mm or more (Gershuni-Gordon & Axer 1974) on the operated side at 2 weeks. After 7 weeks the M.J.S. difference between the two hips had decreased (Table 1).

Indian ink painting

No significant localized patterns of retention of the Indian ink particles were seen on the surface of the articular cartilage on gross inspection, but the painting proved useful from another point of view as will be described later.

Histological sections

Cartilage thickening and flattening was a predominant feature from 2 weeks until 8 weeks postoperatively. After that period cartilage thickening was not found, but in three out of six animals flattening of the head cartilage persisted. The flattening occurred on the superior aspect of the head, sometimes more anteriorly, sometimes more posteriorly (Table 1).

In normal articular cartilage there are chondrocytes dispersed throughout the matrix right up to the surface (Figure 1). On the experimental side death and loss of the superficial layer of the articular chondrocytes was seen, starting in some cases at 2 weeks and becoming almost invariably present between 4 and 5 weeks postoperatively (Figure 2). In some cases the deeper layers of chondrocytes were similarily affected, but the changes were never present exclusively in the deeper layers. In the area of chondrocyte death there were parallel changes both

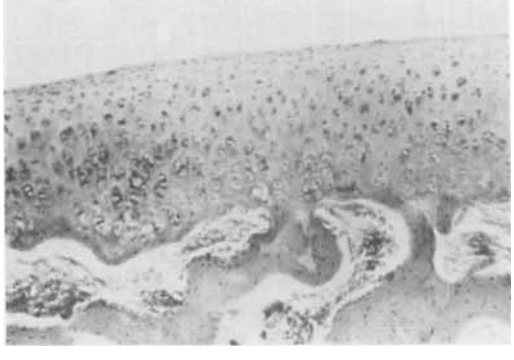


Figure 1. Normal articular cartilage from the femoral head of an 11-week-old rabbit showing regular dispersal of chondrocytes up to the joint surface (Haematoxylin and eosin, × 100).

Table 1. Results of radiological and histological examinations

Time after operation (weeks)	Number of animals	Average difference in M.J.S.* at 2 weeks (mm)	Average difference in M.J.S.* at sacrifice (mm)	Number with cartilage thickening	Number with cartilage flattening	Number with death and loss of cells	Number with loss of G.A.G.s†	Number with cell cloning	Number with fibrillation
2	5	0.9	0.9	4	3	2	1	1	0
3	4	0.9	0.8	3	3	3	3	3	1
4	6	1.1	1.1	5	6	6	6	5	3
5	6	0.7	0.8	5	5	5	3	3	0
6	10	1.0	0.9	7	7	7	6	5	1
7	1	0.5	1.2	1	1	1	1	1	0
8	2	0.9	0.6	1	2	2	2	2	1
9	3	0.9	0.2	0	3	2	0	2	0
10	1	0.5	0	0	0	0	0	0	0
11	2	0.8	0	0	0	1	1	1	0

* M.J.S.—refers to the radiologically measured Medial Joint Space.
† G.A.G.s—Glycosaminoglycans

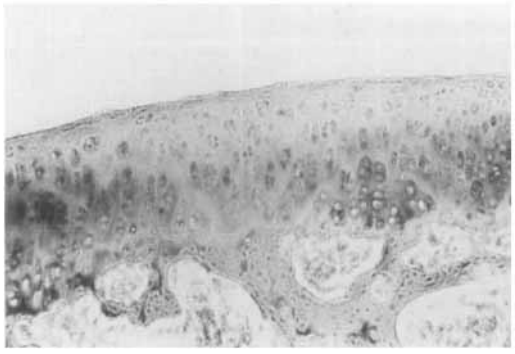


Figure 2. Femoral head articular cartilage 6 weeks after induction of synovitis. There is a decreased cellularity, especially of the superficial cartilage, accompanied by clone formation and decreased metachromasia in the central region of the cartilage (Safranin 0 and fast green, × 100).

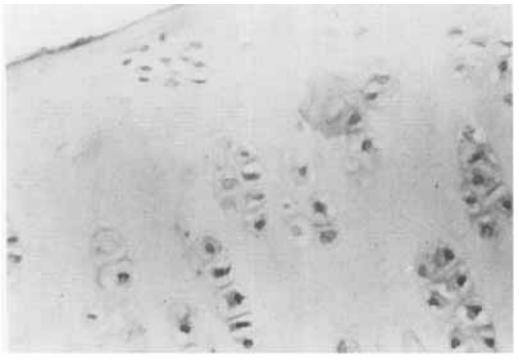
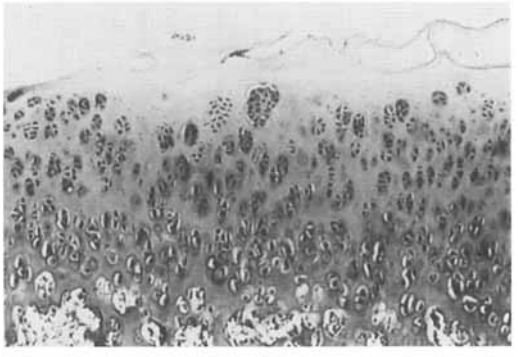


Figure 3. Femoral head articular cartilage 6 weeks after induction of synovitis. The "ghosts" of dead chondrocytes can be seen together with clone formation (Haematoxylin and eosin, × 400).

(a)



(b)

Figure 4. Femoral head articular cartilage 4 weeks after induction of synovitis. (a) There are focal regressive changes with surface fibrillation (Haematoxylin and eosin, × 40). (b) A higher magnification of the fibrillated region showing Indian ink particles on the underside of some collagen fibres. Loss of surface chondrocytes and marked cloning are also well shown (Haematoxylin and eosin, × 100).

topographically and temporally of glycosaminoglycan loss, as evidenced by decreased safranin 0 staining, and of chondrocyte multiplication as shown by clone formation (Figures 2 and 3).

In six cases, three of them at the fourth postoperative week, the surface layers split in a tangential manner denoting early fibrillation. In these cases the Indian ink particles could often be seen on the inferior aspect of the fibrillations confirming that the changes were not artefacts (Figures 4A and 4B). Many of the above changes were still present after 7 weeks, but the tendency was for them to be much less common with increasing time following operation.

The articular cartilage changes occurred in the whole or part of the extent of the flattened area on the superior aspect of the head. In one rabbit killed 6 weeks postoperatively the flattened area of the head showed marked chondrocyte and glycosaminoglycan loss, surface fibrillation and partial collapse of the cartilage into the underlying trabeculae of the bony epiphysis (Figure 5).

The acetabular articular cartilage although thickening *pari passu* with that of the femoral

Figure 5. Femoral head articular cartilage 6 weeks after induction of synovitis. There is fibrillation of the superficial layer with Indian ink particles outlining the disrupted fibres and the underlying cartilage has collapsed into the bony epiphysis. Note also loss of chondrocytes and of uniform metachromasia (Safranin 0 and fast green, × 100).

head did not show such florid changes as just described. Occasionally there were areas of superficial cell loss in a more diffuse manner in the dome region.

None of the above pathological changes were ever seen on the control side.

Radiographic Medial Joint Space (M.J.S.) —Histological correlations

There were 35 animals which maintained an enlarged M.J.S. on the operated side until sacrifice, and their femoral head articular cartilage showed marked histological changes as described above. In five animals that at sacrifice did not show a significant enlargement of the M.J.S., i.e. of 0.3 mm or more (Gershuni-Gordon & Axer 1974), there were no histological changes in three operated hip joints, and in the remaining two cases only slight cartilage thickening and minimal superficial chondrocyte damage were noted.

DISCUSSION

In the human and rabbit hip it has been shown that a very slight natural incongruency allows, on weight-bearing, a better distribution of forces between the two articular cartilages (Bullough et al. 1968, 1973). As soon as this physiological incongruency is disturbed, weight-bearing forces are concentrated over a reduced area, thus increasing the contact stresses.

The present experiment showed that an induced talcum synovitis causes a rapid articular growth response resulting in joint incongruency, as the enlarged femoral head cannot be contained in the less capacious acetabulum. Thus the osteocartilaginous head "grows out" of the acetabulum.

A permanent flattening of the superior aspect of the femoral head cartilage, through which the joint action force passes, was found in most animals. This is explained by the cyclical loading of the decreased area of contact causing the expression of a small, although finite, quantity of water from the underlying matrix (Linn & Sokoloff 1965). If the cyclical loading continues and in addition the area is never completely unloaded, a permanent deformation ensues (Linn 1967), and cartilage nutrition at this spot is interfered with (Gritzka et al. 1973, Maroudas et al. 1968). This results in damage and death of superficial chondrocytes which inhibits repletion of glycosaminoglycans lost in their normal physiological turnover (Mankin & Lippiello 1969). Alternatively, trauma to the chondrocytes releases proteolytic enzymes capable of degrading proteoglycans (Ali 1964, Woessner & Sapolsky 1975). Whichever process is responsible, there is definite histochemical evidence in the present experiment, i.e., the localized loss of safranin 0 uptake (Rosenberg 1971), that the concentration of glycosaminoglycans is diminished in the superficial zone of the articular cartilage of the overstressed area.

Following weakening of the underlying glycosaminoglycan depleted matrix, the increased, mainly radially disposed, cyclical loads caused failure of the horizontal superficial collagen fibres in the articular cartilage (Kempson 1972). Superficial regressive changes with fibrillation occurred only in those cases showing pathological

changes in the deeper layer of the cartilage. In most instances the latter changes were present without accompanying fibrillation, implying that superficial collagen fibre rupture was secondary to glycosaminoglycan loss.

In the area of the femoral head showing regressive cartilage changes there was accompanying chondrocyte multiplication which facilitated reconstitution of the articular cartilage (Mankin & Lippiello 1970). Thus in many of the animals examined at 9 or more weeks after the synovitis insult, the cartilage was nearly normal having presumably repaired itself where remodelling had neutralized the previous incongruency (Gershuni-Gordon & Axer 1974).

An important correlation exists between the degree of enlargement of the radiological M.J.S., which denotes cartilage thickening (Gershuni-Gordon & Axer 1974), and the presence of regressive cartilage changes. This fact connects the severity of cartilage damage directly to the degree of mechanical disturbance developing within the joint.

The findings of the present experimental study may be compared with certain known features of transient synovitis and Legg-Calvé-Perthes' Syndrome (L.C.P.S.) in children's hips. Firstly, there is considerable evidence that L.C.P.S. may be preceded by an episode of synovitis (Fox & Griffin 1956, Jacobs 1960, 1971, Kemp & Boldero 1966 and Spock 1959). The absolute enlargement of the osteochondral elements of the human hip in transient synovitis and L.C.P.S., with the femoral head "growing out" of the acetabulum, has been demonstrated arthrographically (Axer & Schiller 1972, Gershuni et al. 1978). It was also shown that a lateral protrusion of the head and flattening of cartilage in the superior head region occurred in the *early* phase of L.C.P.S. (Gershuni et al. 1978). Larsen & Reimann (1973) and McKibbin & Ralis (1974) noted hyperplasia of the articular cartilage in children with L.C.P.S.; the former authors also described accompanying clone formation and the latter a large macroscopic indentation of the superior femoral head cartilage. One might speculate that if following transient synovitis the same series of changes and damage occurred in the cartilage of the superior aspect of a child's femoral head as was seen in one rabbit's hip (Figure 5), the result of continuing joint action force passing through this decreased weight-bearing area might be to embarrass that component of the capital epiphyseal vasculature traversing the underlying region; a varying degree of bone necrosis could then ensue.

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