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CHONDROCYTE MITOSIS IN THE ARTICULAR CARTILAGE OF FEMORAL HEADS WITH VARIOUS DISEASES

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It is well known that no chondrocytes have mitotic activity in the normal articular cartilage of adult human beings and animals (Mankin 1962 a, b). However, Trias (1961) and Crelin & Southwick (1960, 1964) observed mitotic figures of chondrocytes in the knee joints of adult rabbits under compression. DePalma et al. (1966) demonstrated autoradiographic evidence of thymidine-^{3}H uptake in reparative tissue filled full-thickness defects surgically created. Furthermore, labelled chondrocytes with tritiated thymidine were recognized not merely in autologous diced costal cartilage implanted intramuscularly and intraarticularly (Lemperg 1967) but also in the costal cartilage transplanted to the joint cartilage defects (Hjerquist & Lemperg 1969).

Recently, Swedish and British investigators (Hulth et al. 1972, Telhag 1972, 1973, Rothwell & Bentley 1973) have emphasized that the thymidine-^{3}H uptake can be demonstrated in articular chondrocytes both in experimental animals and in human hips with osteoarthritis.

Although it is acceptable that the degeneration of cartilage may provoke reparative responses including mitosis of chondrocytes, anatomical and metabolic changes in the cartilage matrix in which dividing chondrocytes are embedded are not clearly demonstrated.

The present study is undertaken to answer the question whether or not the mitotic figures of chondrocytes can be found in secondary osteoarthritis and other pathologic conditions of the hip joint and also to reveal the pathologic characteristics of the cartilage matrix showing mitotic figures of chondrocytes.

MATERIALS AND METHODS

Femoral heads were obtained, at the time of operation, from 53 patients with various abnormalities of the hip (Table 1). Small blocks of articular cartilage with

underlying bone were taken from the zenithal area and the medial margin of the joint cartilage under sterile conditions. The specimens were immediately immersed at 36.5° C in Eagle MEM medium* containing 10 microcuries of thymidine-^{3}H (specific activity: 5.0 Ci/mM, the Radiochemical Centre, Amersham, England) per milliliter. After 4–24 hours incubation, specimens were rinsed with physiologic saline solution and fixed in 10 per cent buffered formalin. After decalcification with 10 per cent EDTA solution, dehydration and embedding in paraffin, sections were cut at 6 microns.

Table 1. Number of femoral heads investigated.

Abnormalities		No. of cases
Secondary osteoarthritis		21
Due to congenital dislocation of the hip	20	
Due to spondyloepiphyseal dysplasia	1	
Aseptic necrosis of the femoral head		8
Idiopathic	7	
Posttraumatic	1	
Ununited intracapsular fracture of the femoral neck*		24
Traumatic	20	
^{60}Co irradiated	4	
Total		53

* Femoral heads were obtained from 3 days to 4 years and 4 months after fracture.

Autoradiography was processed by the dipping method. Preparations were exposed in a dark, dry room at 4° C for 4 weeks. After developing and fixation, sections were stained with hematoxylin-eosin. Other sections were stained with either hematoxylin-eosin for cellular details or safranin-O fast green iron hematoxylin for acid glycosaminoglycans (Rosenberg 1971). Since an uncontrolled loss of glycosaminoglycans from the articular cartilage occurs after incubation (Ozerkis & Zarins 1971, Larson & Lemperg 1974), intensity of safranin-O stain in the specimens was carefully judged by comparing it with that in specimens which were taken from the adjacent area and processed without incubation.

Histopathological changes were assessed using the numerical scales of the histological and histochemical grading system described by Mankin et al. (1971).

RESULTS

Chondrocytes labelled with thymidine-^{3}H (Figures 1 & 2) were found in five specimens from the 21 cases with secondary osteoarthritis of the hip, in four from the eight with aseptic necrosis of the femoral

* Eagle MEM medium used is Minimum Essential Medium Eagle with Earle's Balanced Solution.

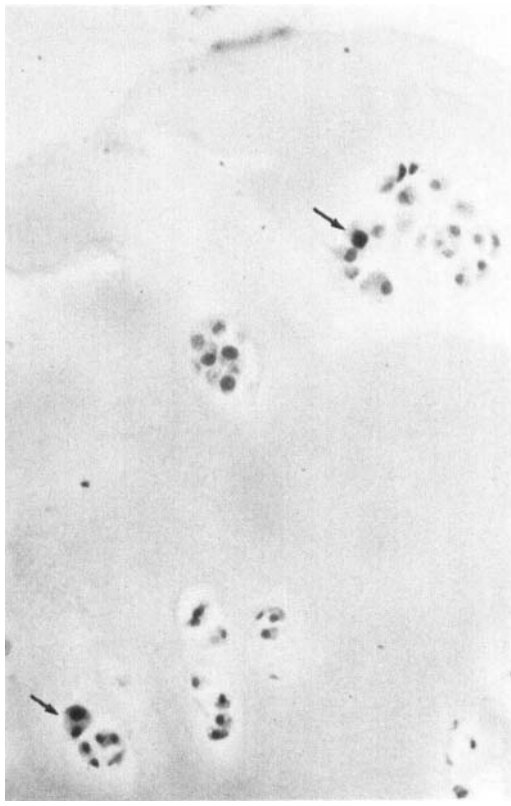

Figure 1. Mitotic chondrocytes in clusters. Arrows indicate the labelled cells (Articular cartilage from a case with secondary osteoarthritis of the hip. × 49

head but in none of the 27 with ununited fracture of the femoral neck. Of all nine specimens containing labelled cells, six were found in the specimens taken from the zenithal area of the head and three from the marginal portion of the cartilage, the incidence being almost the same.

One to four labelled chondrocytes were observed in each specimen. Thymidine-^{3}H was incorporated by either one or two cells within a cluster (Figure 1) or an isolated chondrocyte (Figure 2), the former being more frequently observed. Chondrocytes in the transitional zone of the cartilage exclusively showed a predilection for labelling.

In the cartilage in which mitosis was recognized, safranin-O staining was reduced in the interterritorial matrix with evidence of a positive reaction surrounding chondrocyte lacunae (Figure 3). As shown in Figure 4, histological and histochemical grades of these cartilage specimens ranged from six to ten on their numerical scale. Scores of each criterion are presented in Table 2.

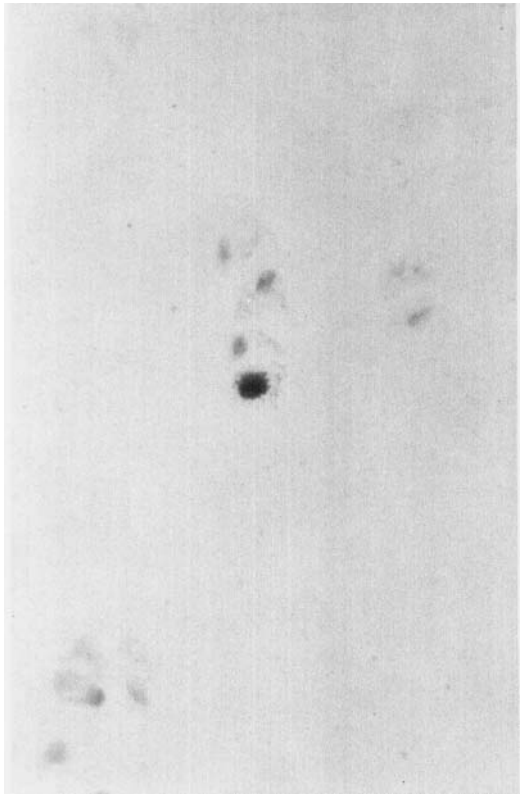

Figure 2. Isolated chondrocyte labelled with tritiated thymidine (Articular cartilage from a case with idiopathic necrosis of the hip. × 163

Table 2. Distribution of scores in nine specimens with mitotic activity.

	Case No.	Histological and histochemical grade				
		I Structure	II Cell	III Safranin-O staining	IV Tidemark integrity	Total
Osteoarthritis;						
Zenith	1	3	2	1	1	7
	2	3	2	3	1	9
	3	4	3	1	1	9
	4	3	3	3	1	10
Margin	5	2	2	3	1	8
Aseptic necrosis;						
Zenith	6	3	2	1	0	6
	7	2	2	2	1	7
Margin	7	2	2	2	1	7
	8	2	2	2	1	7

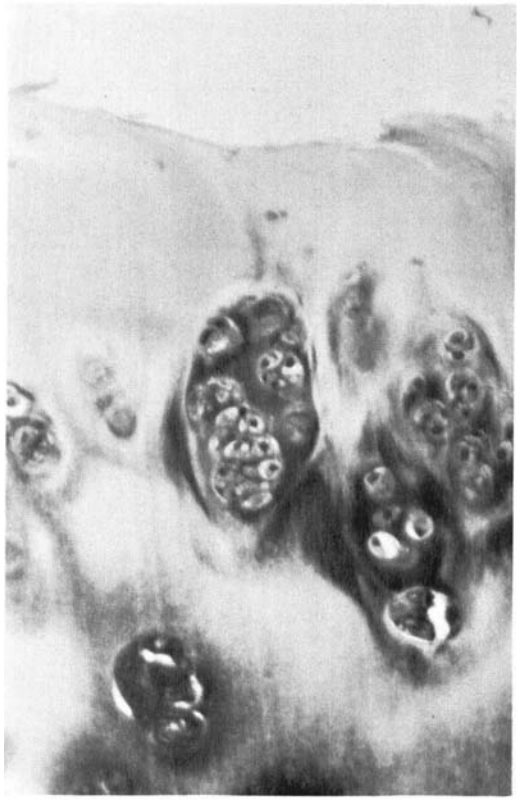

Figure 3. Histology in the area adjacent to Figure 1. Safranin-O fast green stain. × 33

DISCUSSION

The results obtained clearly demonstrated that labelled chondrocytes can be seen in the articular cartilage of both secondary osteoarthritis and aseptic necrosis of the femoral head. It is reasonable to assume that articular chondrocytes in degenerative joint disease restore the mitotic activity and that a hypercellularity and a cluster formation may have resulted from mitotic cell division, as previously stated by Hulth et al. (1972) and Telhag (1972, 1973).

Articular cartilage containing mitotic chondrocytes shows a remarkable degradation of the interterritorial matrix glycosaminoglycans as judged by reduced safranin-O stain. The depletion of matrix glycosaminoglycans may offer a predisposing basis for switching on the DNA synthesis activity of chondrocytes in the mature articular cartilage. The fact that glycosaminoglycans of the matrix are initially degraded in the superficial part of the cartilage supports the finding that the

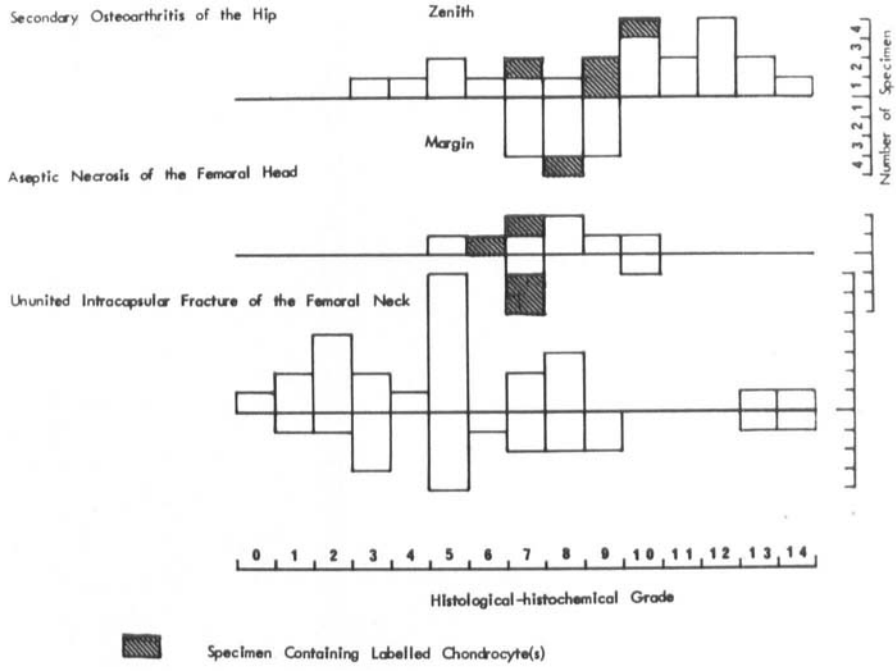

Figure 4. Incidence of mitotic chondrocytes in various diseases of the hip.

labelled chondrocytes are situated in the transitional zone of the cartilige.

The evidence that chondrocytes in the femoral heads of ununited neck fracture show no mitosis leads to a speculation, suggested by previous experiments, that a biomechanical factor seems to play an essential role in provoking mitotic activity of chondrocytes (Trias 1961, Crelin & Southwick 1960, 1964).

The articular cartilage from the heads with aseptic necrosis showed the radioactive thymidine uptake in chondrocytes. In these specimens, subchondral bone was totally dead, the evidence being confirmed by data from another of our investigations using the NADH dehydrogenase reaction (Brücke et al. 1967). The result lends additional support to the concept that the adult articular cartilage is nourished solely by the synovial fluid and is not dependent on the viability of subchondral bone Hodge & McKibbin 1969, Honner & Thompson 1971).

In the present study, histological and histochemical changes are studied to elucidate the anatomical basis responsible for mitosis of chondrocytes. Labelled cells are exclusively found in the degenerated

cartilage which ranged from six to ten on the numerical scale of the histological and histochemical grading system. The results are coincident with that of biochemical studies reported by Mankin et al. (1971). According to them, the thymidine incorporation by degenerated cartilage increased until it reached a score of about ten. A decreased incorporation is observed with increasing scores over that grade. It is possible to state that the reparative process by multiplying chondrocytes by means of mitosis also has "the point of irreversibility" proposed by Mankin (1971). Over that point, complete degradation of the matrix may ensue, finally resulting in chondrocyte death.

SUMMARY

Autoradiographic studies using thymidine-^{3}H reveal the mitosis of chondrocytes in degenerated joints, i.e. joints having secondary osteoarthritis or aseptic necrosis of the femoral head. The findings obtained provide additional support for the recent investigations regarding chondrocyte mitosis in primary osteoarthritic cartilage. Histologic and histochemical examinations suggest that a loss of glycosaminoglycans in the matrix is evidence for conversion of chondrocyte activity to mitosis which occurs, however, within the limit of "the point of irreversibility", analogous to the observations from the biochemical point of view. Biomechanical and nutritional factors are also discussed in relation to the results obtained from cartilages of the femoral heads in cases of femoral neck fracture and aseptic necrosis of the femoral head.

REFERENCES

Brücke, P., Kaufmann, F. & Vagacs, H. (1967) Ergebnisse mit einer neuen histochemischen Methode zur direkten, intraoperativen Bestimmung der Vitalität des Schenkelkopfes bei der medialen Schenkelhalsfraktur. *Arch. orthop. Unfall-Chir.* **62**, 113.

Crelin, E. S. & Southwick, W. O. (1960) Mitosis of chondrocytes induced in the knee joint articular cartilage of adult rabbits. *Yale J. Biol. Med.* **33**, 243.

Crelin, E. S. & Southwick, W. O. (1964) Changes induced by sustained pressure in the knee joint articular cartilage of adult rabbits. *Anat. Rec.* **149**, 113.

DePalma, A. F., McKeever, C. D. & Subin, D. K. (1966) Process of repair of articular cartilage demonstrated by histology and autoradiology with tritiated thymidine. *Clin. Orthop.* **48**, 229.

Hjerquist, S.-O. & Lemperg, R. (1969) Transplantation of autologous costal cartilage to an osteochondral defect on the femoral head. Histological and autoradiographical studies in adult rabbits after administration of ^{35}S-sulphate and ^{3}H-thymidine. *Virchows Arch. path. Anat.* **346**, 345.

Hodge, J. A. & McKibbin, B. (1969) The nutrition of mature and immature cartilage in rabbits. *J. Bone Jt Surg.* **51**-B, 140–147.

Honner, R. & Thompson, R. C. (1971) The nutritional pathways of articular cartilage. *J. Bone Jt Surg.* **53**-A, 742–748.

Hulth, A., Lindberg, L. & Telhag, H. (1972) Mitosis in human osteoarthritic cartilage. *Clin. Orthop.* **84**, 197.

Larson, S.-E. & Lemperg, R. K. (1974) The glycosaminoglycans of the different layers of bovine articular cartilage in relation to age. II. Incorporation of ^{35}S-sulphate in vitro into different fractions of chondroitin sulphate. *Calcif. Tiss. Res.* **15**, 253.

Lemperg, R. K. (1967) Studies of autologous diced costal cartilage transplant. IV. With special regard to ^{3}H-thymidine incorporation in vitro after intramuscular implantation and further transplantation to the hip joint. *Acta Soc. Med. upsalien.* **72**, 199.

Mankin, H. J. (1962 a) Localization of tritiated thymidine in articular cartilage of rabbits. I. Growth in immature cartilage. *J. Bone Jt Surg.* **44**-A, 529.

Mankin, H. J. (1962 b) Localization of tritiated thymidine in articular cartilage of rabbits. III. Mature articular cartilage. *J. Bone Jt Surg.* **44**-A, 682.

Mankin, H. J. (1971) Biochemical and metabolic aspects of osteoarthritis. *Orthop. Clin. N. Amer.* **2**, 9.

Mankin, H. J., Dorfman, H., Lipiello, L. & Zarins, A. (1971) Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hip. II. Correlation of morphology with biochemical and metabolic data. *J. Bone Jt Surg.* **53**-A, 523.

Ozerkis, L. R. & Zarins, A. (1971) Rate of loss of hexosamine from articular cartilage of normal and osteoarthritic human hip. A pilot study. *Bull. Hosp. Jt Dis. (N.Y.)* **32**, 109.

Rosenberg, L. C. (1971) Chemical basis for histological use of safranin-O in the study of articular cartilage. *J. Bone Jt Surg.* **53**-A, 69.

Rothwell, A. G. & Bentley, G. (1973) Chondrocyte multiplication in osteoarthritic articular cartilage. *J. Bone Jt Surg.* **55**-B, 588.

Telhag, H. (1972) Mitosis of chondrocytes in experimental "osteoarthritis" in rabbits. *Clin. Orthop.* **86**, 224.

Telhag, H. (1973) DNA-synthesis in degenerated and normal joint cartilage in full-grown rabbits. *Acta orthop. scand.* **44**, 604.

Trias, A. (1961) Effect of persistent pressure on the articular cartilage. An experimental study. *J. Bone Jt Surg.* **43**-B, 376.

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