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THE BIOCHEMICAL RESPONSE OF RABBIT ARTICULAR CARTILAGE MATRIX TO AN INDUCED TALCUM SYNOVITIS

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Transient synovitis is a commonly occurring affliction in the hips of young children. The results of an experimental model of this condition produced in the immature rabbit's hip showed that there is a significant response in the articular cartilage. Thus, the cartilage rapidly thickens, becomes more hydrated and reveals an initial decrease in glycosaminoglycan concentration. Three weeks following the induction of synovitis, the concentrations of water, glycosaminoglycan and collagen are similar to the control side. The increase of articular cartilage mass is due, not only to an increased hydration, but also to increased matrix production under the stimulus of the synovitis. A similar response by the immature cartilage might be expected following a transient synovitis in a child's hip.

Key words: articular cartilage; hip; synovitis

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The syndrome known as Transient Synovitis commonly occurs in the hip joints of children between the ages of 4 and 8. The condition is not rare (Ferguson 1954) and is said to be responsible for 1 percent of all paediatric admissions (Spock 1959). Although the etiology of this inflammatory condition has not been adequately defined, it has frequently been related to a preceding episode of trauma, a viral or pyogenic infection in a distant part of the body, and finally, hypersensitivity and allergic reactions have been incriminated (Hardinge 1970). With conservative treatment the majority of cases of transient synovitis of the child's hip resolve with no residual stigmata. However, in some cases, an early enlargement of the articular cartilage of the hip joint may be demonstrated (Gershuni et al. 1978) and at a later date Coxa Magna may be seen on plane roentgenography (Ferguson & Howorth 1935). The Coxa Magna can be related to subsequent ossification of the cartilage model enlarged by the effects of the preceding transient synovitis on the immature joint (Gershuni et al. 1978). Finally, a more serious complication of transient synovitis, variously recorded as occurring in 1 to 18 percent of cases, is the subsequent development of radiologically proven Legg-Calvé-Perthes' Syndrome (Spock 1959, Kemp & Boldero 1966, Jacobs 1971).

We have previously shown that a synovitis induced in the immature rabbit hip by the percapsular injection of surgical talcum (magnesium tetrasylicate) is a model for the naturally occurring condition of transient synovitis. The experimental model demonstrated articular cartilage thickening of the femoral head and acetabulum, and consequent incongruency which led to secondary flattening, thinning, and regressive changes of the superior head cartilage (Gershuni-Gordon & Axer 1974, Gershuni et al. 1979).

The present study was designed to answer the question as to whether the cartilage thickening following an induced talcum synovitis was simply

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due to water imbibition or whether the cartilage matrix constituents were altered in a specific and significant fashion.

MATERIALS AND METHODS

Thirty immature New Zealand White rabbits, 1 month-old at the time of operation, were used. The right hip joint capsule was exposed and approximately 0.25 cm^3 of a talcum suspension was injected into the joint as previously described (Gershuni-Gordon & Axer 1974). The left hip served as a control. A further group of six animals underwent an identical procedure except that normal saline was injected into the right hip. The 30 experimental animals were divided into four groups which were sacrificed at respectively 1, 2, 3 and 4 weeks after operation. At 2 weeks after operation and just prior to sacrifice, the hip joints were radiographed in the A.P. projection with the legs in the neutral position. The medial joint space was measured on the radiographs as the horizontal distance between the most medial point on the femoral head and the deepest point of the acetabular floor. After sacrifice, the femoral head articular cartilage, excluding that covering the secondarily flattened superior quadrant and surrounding area, was speedily harvested by a hand-held knife and placed in pre-weighed stoppered bottles. The articular cartilage was analyzed for water, hexosamine, hexuronic acid and hydroxyproline. The saline injected animals were similarly treated 1 week following surgery. The remains of the femoral heads were fixed, decalcified and precisely hemi-sectioned in the vertical plane; corresponding right and left hemi-sections were then simultaneously processed for histological studies. Articular cartilage thickness was measured directly on corresponding hemi-sections from left and right femoral heads, using a microscope eyepiece fitted with a reticle. Thickness measurements were made from three points on the remaining femoral head cartilage and the three measurements were then averaged.

Biochemical analysis

1. *Determination of water.* Water analyses were performed on articular cartilage harvested from the left and right femoral heads. Following re-weighing of the stoppered bottles and contained samples, the cartilage was dried to a constant weight over P_2O_5 in a vacuum for a period of 24 to 48 hours. The amount of water was expressed on a percentage basis.

2. *Hexuronic acid, hexosamine and collagen determinations.* The dried slices of articular cartilage were suspended in 3 ml of 0.15M borate buffer containing 0.02M $CaCl_2$, to which 6 mg of Pronase P was added. The digestion of the articular cartilage was completed after 12 hours at 60°C.

a. *Hexuronic acid:* The hexuronic acid analysis was performed using the automated method of Balazs et al. (1965a). The following modifications were made. In order to reduce the consumption of the sulfuric acid reagent, single acid flex tubes with a capacity of 1.44 ml/min were used to replace two tubes with combined capacity of 1.19 ml/min. The samples from each digest were analyzed directly in triplicate at the rate of twelve samples per hour, each sample being followed by three sample volumes of distilled water.

b. *Hexosamine:* Duplicate aliquots of 0.5 ml from each cartilage digest were placed in tubes fitted with screw caps and Teflon seals. An aliquot (0.5 ml) of concentrated HCl was added to each tube in order to make approximately 6N HCl. The tubes were sealed and hydrolysis was performed at 100°C in a boiling bath for 5 hours. The acid was then removed in a vacuum over P_2O_5 and the dry residue was dissolved in 1 ml of distilled water. The hydrolysates were transferred quantitatively to centrifuge tubes by washing the hydrolysis tubes twice more with 1 ml of water to give a final volume of 3 ml. Activated charcoal was added, the tubes shaken and then allowed to stand for 1 hour before being centrifuged at 2500 rev/min for about 15 minutes. Each clear supernatant was analyzed in triplicate for hexosamine by the automated procedure of Balazs et al. (1965b), utilizing the colorimetric procedure of Elson & Morgan (1933). The hexosamine and hexuronic acid were calculated on the basis of dry weight of cartilage.

c. *Collagen analysis:* Aliquots of 0.5 ml from each cartilage digest were sealed in hydrolysation tubes containing 0.5 ml of concentrated hydrochloric acid. The tubes were then hydrolysed for 3 hours at 130°C and dried in vacuum over P_2O_5. The residues were then redissolved in 10 ml of water after being neutralized and the collagen content was determined on the basis of the hydroxyproline, using the automated procedure of Grant (1964).

RESULTS

The medial joint space of the right hip was enlarged, as measured on the radiographs of the two hips, in all of the four talcum injected groups. The increase was from 0.2 to 0.39 mm which was significant to the 99.5 percent level of confidence at 1 and 2 weeks. The confidence level decreased to the 95 percent level at the 3rd and 4th weeks. There were no differences between the medial joint spaces of the saline injected animals using this technique. The water content of the articular cartilage of the experimental femoral head was significantly increased at 1 and 2 weeks with no significant change at 3 and 4 weeks following

Table 1. Biochemical analysis of articular cartilage following induction of talcum synovitis in the rabbit's hip

Groups	Percent of water		mg per gram of Dry Articular Cartilage: Hexuronic acid		Hexosamine		Collagen	
	Exp.	*Control*	*Exp.*	*Control*	*Exp.*	*Control*	*Exp.*	*Control*
1 week (mean ±S.E.) (7 animals)	76.6±0.7	74.6±1.0	57.2±2.7	63.1±2.9	28.8±1.7	30.4±1.9	455.3± 8.6	459.9± 5.7
Paired *t*-test	$P<0.05$		$P<0.05$		$P<0.05$			
2 week (mean ±S.E.) (8 animals)	79.0±1.2	77.3±1.9	59.9±3.5	63.0±2.0	34.4±1.0	36.8±1.7	476.9±18.9	487.7±18.4
Paired *t*-test	$P<0.05$							
3 week (mean ±S.E.) (7 animals)	78.3±1.4	78.8±2.2	48.5±1.3	46.3±2.1	35.8±2.4	33.7±2.4	482.6±14.2	474.0±14.8
4 week (mean ±S.E.) (8 animals)	78.1±0.8	79.7±1.4	59.6±2.1	62.9±2.5	30.3±3.5	32.7±4.6	476.2±22.1	490.8±21.5
Saline injected 1 week (mean ±S.E.) (7 animals)	79.9±0.6	80.9±1.1	68.7±2.6	70.6±1.5	31.1±0.9	33.2±1.3	399.6±11.8	411.0± 6.1

Where *t*-test not indicated the result is not statistically significant.

operation. Concentrations of hexosamine and hexuronic acid decreased significantly as measured on the dried samples at 1 week. At 2 weeks the values were still reduced, but the differences were not statistically significant. They showed no differences at 3 and 4 weeks following induction of synovitis. There were relatively small decreases of collagen concentration on the experimental side at 1 and 2 weeks, which by statistical testing were not significant. There were no differences in collagen concentration 3 and 4 weeks after injection. In the saline injected animals, a questionably significant decrease of hexosamine concentration was found on the experimental side but there were no changes in water or collagen concentration (Table 1).

Femoral head articular cartilage thickness was shown to be increased on the talc injected side at the 1, 2, 3 and 4 postoperative weeks. The difference ranged between 13–23 percent and was highly significant ($P < 0.025$). In the saline injected animals there was no difference in cartilage thickness between the two sides.

DISCUSSION

Previous work has shown that following the induction of a talcum synovitis in the hip joint of the rabbit, the radiological medial joint space enlarged although the size of the osseous femoral capital epiphysis paralleled that of the control side (Gershuni-Gordon & Axer 1974). When the enlargement of the space on the experimental side exceeded that of the control side by 0.3 mm, it correlated with thickening of articular cartilage, resulting in a larger osteochondral femoral head (and a relatively smaller acetabular capacity). Direct measurement in the present experiment confirmed the thickening of the cartilage in cases where previous evidence of enlargement of the radiological medial joint space had been obtained. The mass of femoral head articular cartilage thus increased in an absolute fashion from the first week following induction of the talcum synovitis. The increase in cartilage thickness was of the order of 15 percent as compared to the control side; a factor greatly in excess of the 3 percent increase in hydration. It is concluded that

the immature articular cartilage rapidly responded to the synovial inflammation. Presumably the latter, by causing local increase of temperature, hyperemia and venous congestion, facilitated anabolic cellular activity and hence, increased production of cartilage matrix (Hutchinson & Bordeaux 1954, Richards & Stofer 1959, McKibbin & Holdsworth 1966).

The injection of saline alone in the rabbits' hips produced no measurable radiological or histological change, but this minor insult to the joint provoked a slight change in hexosamine concentration. Other authors have also found that simply opening the knee joint of rabbits caused increased uptake of 35S-sulfate (Meachin 1964), increased collagen (Floman et al. 1978) and glycosaminoglycan production (Mankin 1964) by the articular cartilage. It may be inferred that any invasive procedure of a synovial joint may, of itself, produce biochemical changes which must be taken into consideration in assessing the results of any experimentation on such joints.

The effects of the induced synovitis were not simply increased hydration of the articular cartilage and corresponding dilution of the matrix constituents. Thus, in the animals examined 1 week following the operation, an increased water content was accompanied by a decrease in glycosaminoglycan concentration but there was no significant change in collagen concentration. Three and four weeks following operation, the cartilage constituents of the right and left hips showed no significant differences. Thus, the synovitis effect had disappeared and the newly formed, *yet thicker,* cartilage on the experimental side had matured to resemble that on the control side. Because the cartilage mass increased by a factor exceeding the increased hydration, more glycosaminoglycan and collagen must have been formed. It has been noted that in the rabbit at 2 months of age, chondrocytes in articular cartilage are most numerous, are continually replicating and are extremely active in synthesizing proteins (Mankin 1964, Mankin & Baron 1965). The present experiment demonstrated that, additionally, the chondrocytes of immature cartilage have the capability to respond to appropriate stimuli and to synthesize proteoglycan and collagen at an even faster rate than normal.

It has been suggested that collagen may attract more water and be a more effective gel-former than proteoglycan (Mankin & Thrasher 1975). In addition, newly formed collagen, prior to the development of cross links, may more avidly bind water molecules. If the newly synthesized proteoglycan (and collagen) molecules are of smaller than normal size (Hardingham et al. 1972), the resulting increased colloid osmotic pressure of the cartilage would provoke an increased hydration (Bollett & Nance 1966), as was found 1 and 2 weeks after synovitis induction. As the molecular structure matures, aggregation of hyaluronic acid and proteoglycan molecules occurs (Hardingham et al. 1972), the osmotic pressure returns to a more normal level and the cartilage is thus less hydrophilic than in the early synovitis stage (McDevitt & Muir 1976).

It was thus found than an induced synovitis in the hip joint of the rabbit can produce a distinct and significant change in both the cartilage thickness and its matrix constituents. If the same sequence of events were to occur in the hip of a young child, it is clear that a so-called Transient Synovitis may not be the benign condition it is often labeled, but may have damaging sequelae. These experimental results also provide an explanation for the development of Coxa Magna following hip Transient Synovitis (Ferguson & Howorth 1935) and Legg-Calvé-Perthes' Syndrome (Schiller & Axer 1972, Gershuni et al. 1978).

REFERENCES

Balazs, E. A., Bernstein, A. O., Karossa, J. & Swan, D. A. (1965a) An automated method for the determination of hexuronic acids. *Anal. Biochem.* **12**, 547.

Balazs, E. A., Bernstein, A. O., Karossa, J. & Swan, D. A. (1965b) An automated method for the determination of hexosamine. *Anal. Biochem.* **12**, 559.

Bollett, A. J. & Nance, J. L. (1966) Biochemical findings in normal and osteoarthritic articular cartilage II. Chondroitin sulfate concentration and chain length, water and ash content. *J. Clin. Invest.* **45**, 1170.

Elson, L. A. & Morgan, W. T. (1933) Colorimetric method for the determination of glucosamine and galactosamine. *Biochem J.* **27**, 1824.

Ferguson, A. B. & Howorth, M. B. (1935) Coxa Magna. *JAMA.* **104**, 808.

Ferguson, A. B. (1954) Synovitis of the hip and Legg-Perthes' disease. *Clin. Orthop.* **4**, 180.

Floman, Y., Eyre, D. R., Shapiro, F. D. & Glimcher, M. J. (1978) Altered biosynthesis of collagen and proteoglycan by cartilage in early experimental osteoarthrosis in the rabbit. *Trans. Orthop. Res. Soc.* **3**, 136.

Gershuni-Gordon, D. H. & Axer, A. (1974) Synovitis of the hip joint. An experimental model in rabbits. *J. Bone Joint Surg.* **56**-B, 69.

Gershuni, D. H., Axer, A. & Hendel, D. (1978) Arthrographic findings in Legg-Calvé-Perthes' disease and Transient Syndrome of the hip. *J. Bone Joint Surg.* **60**-A, 457.

Gershuni, D. H., Axer, A. & Siegel, B. (1979) Localized regressive articular cartilage changes in the hip joint of the rabbit following an induced synovitis. *Acta Orthop. Scand.* **50**, 179.

Grant, R. A. (1964) Application of the auto-analyzer to connective tissue analysis. The determination of hydroxyproline and hexosamines. Technicon International Symposium, London, England.

Hardinge, K. (1970) The aetiology of Transient Synovitis of the hip in childhood. *J. Bone Joint Surg.* **52**-B, 100.

Hardingham, T. E., Fitton-Jackson, S. & Muir, H. (1972) Replacement of proteoglycans in embryonic chick cartilage in organ culture after treatment with testicular hyaluronidase. *Biochem. J.* **129**, 101.

Hutchinson, W. J. & Bordeaux, B. D. (1954) The influence of stasis on bone growth. *Surg. Gynecol. Obstet.* **99**, 413.

Jacobs, B. W. (1971) Synovitis of the hip in children and its significance. *Pediatrics* **47**, 558.

Kemp, H. S. & Boldero, J. L. (1966) Radiological changes in Perthes' disease. *Br. J. Radiol.* **39**, 744.

Mankin, H. J. (1964) Mitosis in articular cartilage of immature rabbits. *Clin. Orthop.* **34**, 170.

Mankin, H. J. & Baron, P. A. (1965) The effect of aging on protein synthesis in articular cartilage of rabbits. *Lab. Invest.* **14**, 648.

Mankin, H. J. & Thrasher, A. Z. (1975) Water content and binding in normal and osteoarthritic human articular cartilage. *J. Bone Joint Surg.* **57**-A, 76.

McDevitt, C. & Muir, H. (1976) Biochemical changes in the cartilage of the knee in experimental and natural osteoarthritis in the dog. *J. Bone Joint Surg.* **58**-B, 94.

McKibbin, B. & Holdsworth, F. W. (1966) The nutrition of immature joint cartilage in the lamb. *J. Bone Joint Surg.* **48**-B, 793.

Meachin, G. (1964) Sulphate metabolism of articular cartilage after surgical interference with the joint. *Am. Rheum. Dis.* **23**, 372.

Richards, V. & Stofer, R. (1959) The stimulation of bone growth by internal heating. *Surgery* **46**, 84.

Schiller, M. G. & Axer, A. (1972) Hypertrophy of the femoral head in Legg-Calvé-Perthes' Syndrome (L.C.P.S.). *Acta Orthop. Scand.* **43**, 45.

Spock, A. (1959) Transient Synovitis of the hip joint in children. *Pediatrics* **24**, 1042.

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