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Metabolic responses of cartilage in experimentally induced osteoarthritis

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SUMMARY Serial metabolic responses in developing osteoarthritis induced in the right knees of a rabbit model of partial meniscectomy (PM) were studied. Controls were sham-operated (SH) right knees, left knees of all operated animals, and right and left knees of a nonoperated series. Glycosaminoglycan and protein synthesis and cell replication were separately analysed utilising $^{35}SO_4$, ^{14}C-glycine, and ^{3}H-thymidine, respectively. Pitting, ulceration, and osteophytes, seen only in the PM knees, increased over the 12-week period of study. ^{3}H-thymidine and ^{14}C-glycine incorporations by PM cartilage were increased at 3 weeks, less than nonoperated control animals at 9 weeks, and approximated to those of controls at 12 weeks. $^{35}SO_4$ incorporation by PM cartilage was decreased throughout the 12 weeks. Similar patterns were noted in sham-operated knees. $^{35}SO_4$ incorporation by tibial osteophytes was decreased at 9 and 12 weeks. Similar isotope incorporations seen after partial meniscectomy and sham surgery represented a nonspecific response to arthrotomy. Cartilage synthetic activity did not increase in parallel with degenerative change.

The metabolic responses of cartilage which take place during the development of osteoarthritis have been the subject of a number of investigations over the past several decades.[1–10] Early studies by Collins and McElligott in human osteoarthritis[2] revealed an increased $^{35}SO_4$ incorporation. Further studies in man demonstrated significant increases in the rates of incorporation of ^{3}H-thymidine, ^{14}C-glycine, and $^{35}SO_4$ in osteoarthritic cartilage.[6] The rates of ^{3}H-thymidine and $^{35}SO_4$ incorporation increased in a nonlinear fashion up to a certain level of disease activity.[5] At a certain point in the osteoarthritic process, however, reparative cellular activity and proteoglycan synthesis appeared to fail, leading to progressive cartilage destruction. Similar observations were reported in studies by Thompson and Oegema.[10] Early stages of osteoarthritic disease were characterised by an increased rate of glycosaminoglycan synthesis; in late stages of disease the rate of synthesis was decreased. Opposite but conflicting observations have been noted by other investigators. Maroudas[7] showed that, when human articular cartilage was severely fibrillated, $^{35}SO_4$ incorporation was often lower than in normal cartilage; in mildly fibrillated cartilage no differences from normal cartilage were noted. McKenzie *et al.*[9] showed that rates of incorporation of $^{35}SO_4$ into glycosaminoglycans of specimens of human osteoarthritic cartilage were independent of grading severity. These latter findings are in agreement with those of Bollet and Nance.[1]

Metabolic studies of the response of cartilage to osteoarthritic breakdown have also been performed in experimental models. Mayor and Moskowitz,[8] using autoradiographic studies in a rabbit model of experimental osteoarthritis, found increases in cartilage protein and glycosaminoglycan synthesis and in cell replication which closely paralleled pathological changes in cartilage. Ehrlich and coworkers,[3] using a different model of experimental osteoarthritis in the rabbit,[11] demonstrated a significant increase in the rates of synthesis of protein and proteoglycan. However, tritiated thymidine incorporation was not significantly increased. These findings in the rabbit contrast with the observations of Lust *et al.*[4] in a canine model, in which overall synthetic activities for DNA, protein, and glycosaminoglycan were decreased in osteoarthritic lesions as compared with normal areas of cartilage.

An understanding of the pathophysiology of osteoarthritis requires resolution of the contrasting

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observations noted above. A number of factors complicate delineation of these mechanisms in studies on human cartilage. Among these factors are differences in sampling of cartilage undergoing variable amounts of pathological change in the same specimen, difficulties in standardising and grading pathological change,[5] inaccuracy in quantitation of disease duration, and the inability to define results in specimens of cartilage undergoing degenerative change at serial timed intervals during the development of disease. Experimental models of osteoarthritis obviate several of these factors, notably the ability to define disease duration and to obtain serial specimens for longitudinal tissue analysis. In the present study serial metabolic responses in cartilage undergoing osteoarthritic change in an experimental animal model are defined.

Methods and materials

Knee joints from 3 groups of immature New Zealand white female rabbits were studied (Table 1). In the first group of 19 animals (group I) degenerative lesions were induced in the right knees by the method of partial medial meniscectomy.[12] Left knees, unoperated upon, were evaluated for control. In a second group of 15 animals (group II) an identical surgical procedure to partial medial meniscectomy was performed, except that no meniscal tissue was excised (sham-operated knees). Left knees, unoperated upon, were evaluated for control. In a third group of 12 animals (group III) right and left knees of rabbits in which no surgery was performed on either knee were separately evaluated. Surgical procedures in the first 2 groups were performed under an analgesic/anaesthetic technique in which ketamine HCl 35 mg/kg and xylazine 5 mg/kg were given intramuscularly, followed by local infiltration of the surgical site with 2% lignocaine plus 1:100 000 adrenaline.

The average initial weight for animals in group I was 2·1 kg (range 1·8–2·7 kg), and in group II, 2·1 kg (range 1·6–2·3 kg). The average final weight for animals in group I was 3·2 kg (range 2·2–4·6 kg) and in group II, 3·0 kg (range 1·8–4·0 kg). Group III control animals comprised 2 different weights, so as to bracket the animal weights of lighter operated animals killed at 3 weeks and heavier operated animals killed at 12 weeks. Accordingly 6 nonoperated animals of average weight 2·4 kg (range 2·2–2·6 kg) and 6 animals of average weight 4·0 kg (range 3·7–4·2 kg) were studied. Animals in groups I and II were killed at intervals of 3, 4, 6, 9, and 12 weeks after surgery. Four animals were killed at each time period in the partial meniscectomy series, except for the 3-week series which contained only 3 animals. In the sham-operated series 3 animals were killed at each time period, except for the 3-week and 6-week series, in which 2 and 4 animals were included, respectively.

Table 1 *Outline of study groups as defined by surgical procedures in right and left knees undergoing analysis*

	Group I partial meniscectomy animals	*Group II sham-operated animals*	*Group III nonoperated animals*
Right knee	Operated	Operated	Nonoperated
Left knee	Nonoperated	Nonoperated	Nonoperated

After aseptic preparation of skin and separation of underlying muscle and capsular tissue the femoral and tibial articular surfaces of the knee were exposed and debrided of noncartilaginous tissue. Femoral and tibial surfaces were examined grossly for evidence of pitting and ulceration of cartilage, and for osteophyte formation. Distal femurs and proximal tibias were separated with bone cutters, and gross findings recorded as to extent and severity of lesions. Sketches of the location of lesions were made for each specimen. Disarticulated specimens were placed in Gey's balanced salt solution containing penicillin-streptomycin 1%, Fungizone (amphotericin B) 1%, and Mycostatin (nystatin) 0·1%.

Sterile debridement of specimens was now performed to completely remove noncartilaginous soft tissues from contiguous areas of the cartilage surface from each specimen. Cartilage from medial femoral condyle, lateral femoral condyle, medial tibial plateau, and lateral tibial plateau quadrants and from tibial osteophytes was separately removed tangential to the joint surface, and it was combined in each group, and at each sacrifice time according to anatomical location to allow analysis of cartilage by specific joint area. Sufficient cartilage was available from animals at each sacrifice interval so as to provide material for triplicate analysis of ^{3}H-thymidine, $^{35}SO_4$ and ^{14}C-glycine incorporation (see below). In the case of the nonoperated control animals, 6 each were studied at the 2·4 and 4·0 kg average weight ranges. Cartilage from each set of 3 animals was combined, thereby providing 2 sets of cartilage for triplicate isotope analysis at each control weight range.

Cartilage slices were exposed to 0·05% testicular hyaluronidase for 3 minutes to digest contaminating blood cells and synovial fluid. All cartilage was subsequently rinsed in 2 separate washes of Gey's balanced salt solution, chopped finely into pieces measuring 0·4 mm or less, and then divided into 9 approximately equal portions. These aliquots were transferred to microtest plates (Falcon

Plastics) to which was added 1 ml Dulbecco's modified Eagle's medium (4·5 g/l glucose) supplemented with fetal bovine serum 10%, penicillin-streptomycin 0·1%, Fungizone 1%, and Mycostatin 0·1%. Specimens were incubated for 20–22 h in an atmosphere of 10% CO_2 in air at 37°C.

After preincubation the media were removed and new media added which separately contained $Na_2\,^{35}SO_4$, 1·4 μCi/ml or ^{14}C-glycine, 1 μCi/ml. $^{35}SO_4$ incorporation experiments were performed in Dulbecco's modified Eagle's medium further modified by removal of $MgSO_4$ and replacement with $MgCl_2$ (165 mg/l). ^{14}C-glycine was used to label the total newly synthesised protein in Eagle's minimal essential medium devoid of endogenous glycine.

^{3}H-thymidine (1 μCi/ml) was added directly from a stock solution without any change of medium. This procedure was developed so as to minimise the contribution of fresh serum to ^{3}H-thymidine incorporation. All media contained 10% fetal bovine serum and supplements as above. Incubation of cartilage was carried out for 20 h at 37°C, after which the reaction was stopped by removal of medium. Media from $^{35}SO_4$ and ^{14}C-glycine experiments were removed and frozen until analysed. Tissues were washed in Dulbecco's phosphate buffered saline (DPBS) pH 6·9 once and incubated in a third wash at 37°C for 45 minutes. The media from ^{3}H-thymidine labelling were discarded. The tissues were washed once in DPBS and incubated in DPBS containing 5 μM thymidine at 37°C for 45 minutes.[13] Tissue-insoluble material was precipitated with ice-cold 10% TCA for 15 minutes, followed by hydrolysis of tissues in alkali overnight at 37°C. Radioactivity was counted on an aliquot of the tissue hydrolysate and the remainder used to analyse tissue protein by the method of Lowry *et al.*[14] Radioactivity in the form of sulphated-GAG ($^{35}SO_4$) or protein (^{14}C-glycine) secreted into the medium was analysed by dialysis against 12 000 MW cut-off membranes (A. H. Thomas, Philadelphia, PA). $^{35}SO_4$ samples were dialysed against 0·075 M $(NH_4)_2SO_4$ for 4 h at 4°C followed by cold running tap water overnight. ^{14}C-glycine samples were dialysed against cold running tap water only. Isotope incorporations were analysed as dpm/mg protein (disintegrations per minute).

Technical considerations precluded simultaneous assay of isotope incorporation and DNA content on the same tissue samples. Accordingly isotope incorporations were analysed as dpm/mg protein, as noted. In order to assess the validity of expressing analyses in this manner, ratios of μg DNA/mg protein in normal and osteoarthritic rabbit cartilage were determined and compared. Cartilage from femurs and tibias of 3 normal rabbits, and from 3 rabbits killed 12 weeks after partial meniscectomy were studied by procedures described above. Tissues were combined by quadrant (medial femoral condyle, lateral femoral condyle, medial tibial plateau, and lateral tibial plateau). Tibial osteophyte tissue, found only in the osteoarthritic partial meniscectomy knees, was also studied. Total cartilage samples were divided into 2 approximately equal aliquots and wet weights determined. DNA content[15] and total protein were separately analysed. Data were expressed as μg DNA/mg protein.

Statistical analyses were performed by paired *t* tests for intra-animal comparisons, and independent group *t* tests for comparisons among groups of animals.[16]

Results

PATHOLOGICAL FINDINGS

Gross pathological abnormalities were seen only in the knees in which partial medial meniscectomy had been performed (Table 2). No abnormalities were noted at 3 or 4 week intervals. Femoral pitting was maximal at 9 and 12 weeks; femoral ulceration increased over time, with maximal percentage of animals showing ulcerations at 12 weeks. Small femoral spurs were noted in 1 animal each at the 6 and 9 week time periods. No tibial pitting or ulcerations were seen. Osteophyte formation along the entire edge of the medial tibial plateau was almost universal in animals killed at 6, 9, and 12 weeks.

Table 2 *Gross pathological findings at time of death in animals subjected to partial meniscectomy*

Partial meniscectomy right knees (wks)	*No.*	*Femur*			*Tibia*		
		Pitting	*Ulcers*	*Osteophytes*	*Pitting*	*Ulcers*	*Osteophytes*
		No. (%)	*No.* (%)	*No.* (%)–size (mm)	*No.* (%)	*No.* (%)	*No.* (%)–size (mm)
3	3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4	4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
6	4	2 (50)	1 (25)	1 (25)–0·5 mm	0 (0)	0 (0)	4 (100)–2·2 mm
9	4	3 (75)	2 (50)	1 (25)–0·5 mm	0 (0)	0 (0)	3 (75)–1·5 mm
12	4	3 (75)	3 (75)	0 (0)	0 (0)	0 (0)	4 (100)–2·4 mm

METABOLIC FINDINGS

DNA/protein ratios. In normal and partial meniscectomy osteoarthritic cartilage (Table 3) these were similar when corresponding quadrants from which cartilage was obtained were compared. Femoral cartilage ratios were consistently higher than tibial cartilage ratios. The highest ratio was noted in medial tibial osteophyte tissue.

Non-operated animals. Isotope incorporations were reproducibly similar in the 2 sets of 3 animals each studied at the 2·4 kg and the 4·0 kg average weight ranges (2-tailed independent groups *t* test, $p<0·05$). Accordingly the 2 sets of data were combined for analysis.

Numerical values for incorporation of all 3 isotopes were consistently greater in femoral cartilage than in opposing tibial cartilage (Table 4). Similarly isotope incorporation into lateral tibial plateau cartilage exceeded that in the medial tibial plateau except for sulphate incorporation in the right knees, in which case medial and lateral tibial incorporations were identical. Comparison of isotope incorporations into medial femoral versus lateral femoral cartilage revealed essentially no differences except for thymidine incorporation in the right knees and sulphate incorporation in the left knees. Comparisons in which numerical differences were shown to be statistically significant are noted in Table 5 (2-tailed paired *t* test significant, $p<0·05$, DF = 3).

Tissue/media partition data. No statistically significant differences in partitioning of isotope incorporation between partial-meniscectomy and sham-operated animals were noted when tissue incorporation (DPM_{tiss}) was compared with total incorporation into tissue plus media ($DPM_{tiss} + DPM_{med}$) (Table 6). ^{14}C-glycine incorporation at 3 weeks was reduced as compared with data derived at weeks 4, 6, 9, and 12. Although incorporation ratios for tibial osteophytes were slightly lower than the median ratios for other surfaces within the same knee, the differences were not statistically significant.

Table 3 *Ratios of μg DNA/mg protein in normal and osteoarthritic knee cartilage by quadrant location*

	Normal knee cartilage	*OA knee cartilage*
MFC*	0·020	0·019
LFC	0·021	0·020
Average	*0·02050*	*0·0195*
MTP	0·015	0·013
LTP	0·011	0·014
Average	*0·0130*	*0·0135*
MTO	—	0·039

*M = medial; L = lateral; F = femoral; T = tibial; C = condyle; P = plateau; O = osteophyte.

Table 4 *Isotope incorporation in nonoperated control animals (group III)*

	*^{3}H-thymidine**	*$^{35}SO_4$**	*^{14}C-Glycine**
Right knees			
MFC†	6·0±0·5	34±3	42±2
LFC	8·5±0·7	33±6	40±3
MTP	3·0±0·6	22±7	17±1
LTP	5·1±0·6	22±2	32±4
Left knees			
MFC	6·0±0·6	35±6	44±4
LFC	5·8±0·7	30±3	43±4
MTP	3·1±0·4	13±2	17±1
LTP	4·4±1·3	20±2	28±5

*DPM/mg protein × 10^{-4} ± SE.
†M = medial; L = lateral; F = femoral; T = tibial; C = condyle; P = plateau.

Table 5 *Statistically significant differences in isotope incorporation observed when various intra-articular surfaces in group III nonoperated control animals were compared*

Isotope	*Site*	*Significant comparison*
^{3}H-thymidine	Right knee	MFC>MTP* LFC>LTP
	Left knee	MFC>MTP LFC>LTP
$^{35}SO_4$	Right knee	LFC>LTP
	Left knee	MFC>MTP LTP>MTP
^{14}C-glycine	Right knee	MFC>MTP LTP>MTP
	Left knee	MFC>MTP LFC>LTP

*M = medial; L = lateral; F = femoral; T = tibial; C = condyle; P = plateau.

Table 6 *Median ratios of isotope incorporation into tissue (DPM_{tiss}) compared to isotope incorporation into tissue plus media ($DPM_{tiss} + DPM_{med}$) for medial and lateral femoral condyles and tibial plateaus for each knee. Differences between partial-meniscectomy and sham-operated animals were not statistically significant by paired* t *test.*

Group	*Week killed*	*^{14}C-Glycine*		*$^{35}SO_4$*	
		Right knee	*Left knee*	*Right knee*	*Left knee*
Partial-meniscectomy animals (group I)	3	0·49	0·53	0·89	0·82
	4	0·80	0·80	0·90	0·89
	6	0·79	0·75	0·89	0·91
	9	0·84	0·82	0·76	0·90
	12	0·80	0·81	0·92	0·91
Sham-operated animals (group II)	3	0·57	0·45	0·89	0·90
	4	0·80	0·80	0·90	0·90
	6	0·80	0·80	0·90	0·90
	9	0·71	0·81	0·91	0·92
	12	0·79	0·81	0·89	0·90

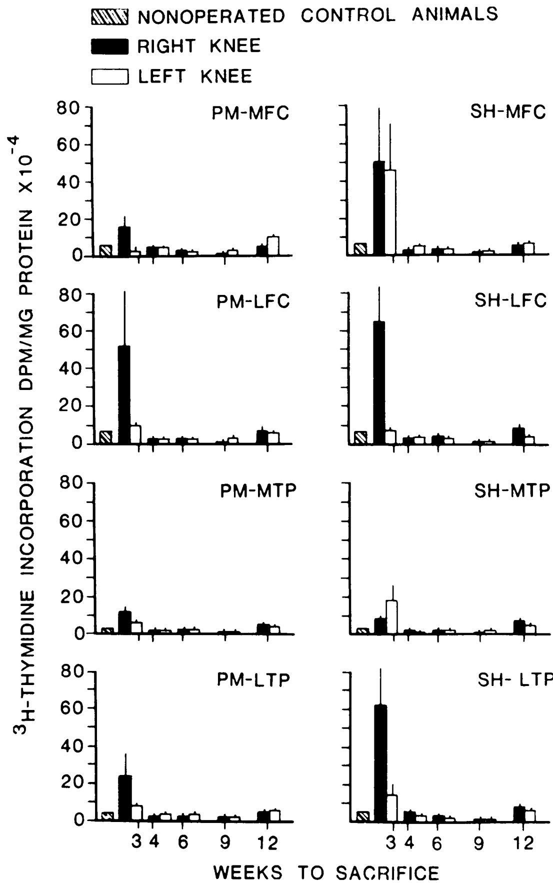

Fig. 1 *^{3}H-thymidine incorporation into various surfaces of right and left knees of partial meniscectomy (PM) and sham-operated (SH) animals when killed. Isotope incorporation into knees of nonoperated animals (group III) is given at the extreme left of each graph as baseline control. M = medial; L = lateral, F = femoral; T = tibia; C = condyle; P = plateau.*

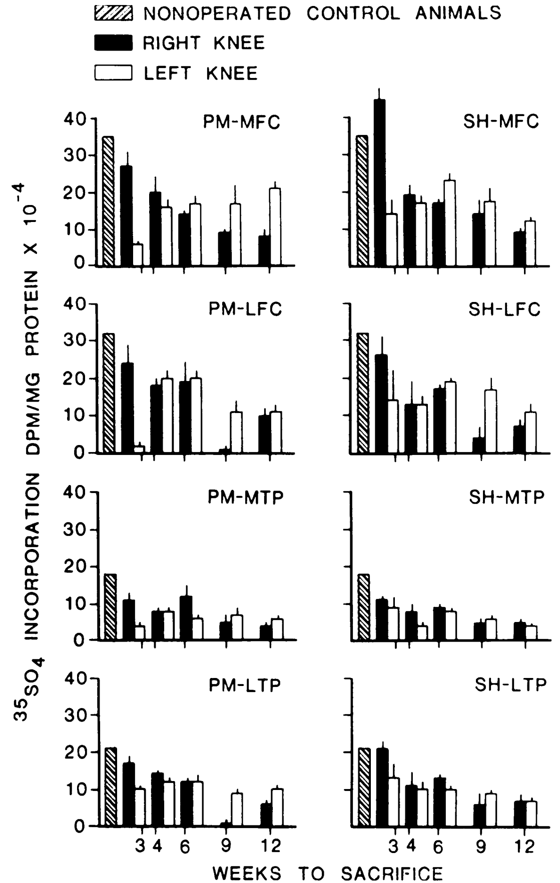

Fig. 2 *$^{35}SO_4$-sulphate incorporation into various surfaces of right and left knees of partial meniscectomy (PM) and sham-operated (SH) animals when killed. Isotope incorporation into knees of nonoperated animals (group III) is given at the extreme left of each graph as baseline control. M = medial; L = lateral, F = femoral; T = tibia; C = condyle; P = plateau.*

Partial meniscectomy animals. (a) ^{3}H-thymidine incorporation by cartilage of right knees was increased at 3 weeks (Fig. 1). Incorporation then fell to levels lower than those seen in control nonoperated animals at 9 weeks, followed by a return toward control levels in nonoperated animals at 12 weeks. A similar pattern of incorporation was observed in the nonoperated left knees of the same animals, although the increase at 3 weeks was generally less marked.

(b) $^{35}SO_4$ incorporation by right knee cartilage was decreased in comparison with nonoperated control animals over the time periods studied, with the lowest incorporations observed at 9 and 12 weeks (Fig. 2). Although the uptake pattern observed in the nonoperated left knees showed a similar trend, sulphate incorporation at 3 weeks showed a sharp decline in all the surfaces studied, and at 12 weeks incorporation of isotope by cartilage in the medial femoral condyle showed some return towards the level seen in nonoperated control animals.

(c) ^{14}C-glycine incorporation: Like the findings with ^{3}H-thymidine, incorporation by right knee cartilage was increased at 3 weeks compared with nonoperated control animals, followed by a progressive fall to levels less than nonoperated controls at 9 weeks (Fig. 3). A return of incorporation to values approaching levels seen in nonoperated

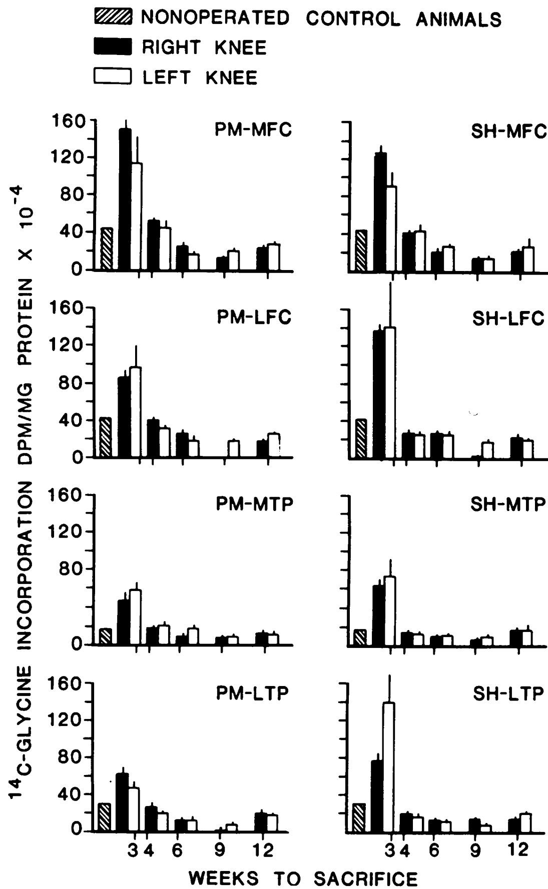

Fig. 3 *^{14}C-glycine incorporation into various surfaces of right and left knees of partial meniscectomy (PM) and sham-operated (SH) animals when killed. Isotope incorporation into knees of nonoperated animals (group III) is given at the extreme left of each graph as baseline control. M=medial; L=lateral, F=femoral; T=tibia; C=condyle; P=plateau.*

control animals was noted at 12 weeks. An almost identical pattern of incorporation was noted in nonoperated left knees.

Sham-operated snimals. Incorporation patterns for all 3 isotopes (Figs 1, 2, 3) were similar to patterns described in the partial meniscectomy animals for both operated right and nonoperated left knees.

Osteophytes. Isotope incorporation by osteophyte tissue was compared with that of contiguous medial tibial plateau (MTP) cartilage of the same knee (Fig. 4). Statistically significant differences were observed only with thymidine at 9 and 12 weeks, and these differences were inconsistent in direction. When osteophyte isotope incorporation was compared with that of medial tibial plateau cartilage from nonoperated control animals, a statistically significant decrease in sulphate incorporation was observed at 9 and 12 weeks, and in glycine incorporation at 9 weeks in the osteophytic tissue.

Discussion

Isotope incorporation patterns by cartilage from partially meniscectomised and sham-operated knees of rabbits were similar and appear to represent a nonspecific response to pathophysiological changes associated with arthrotomy. Cell replication and protein synthesis were stimulated early, followed by a decrease to subnormal values at later times of sacrifice. By 12 weeks these metabolic parameters were at or approached normal. Proteoglycan synthesis, as measured by sulphate incorporation, was generally inhibited throughout the entire 12-week time span of study. Metabolic assessments were not performed with any of the isotopes prior to the 3-week interval postoperatively. Accordingly findings similar to those observed at 3 weeks may well have been present immediately or soon after arthrotomy was carried out. Such findings, if present, would support the nonspecific nature of the response, unrelated to partial meniscectomy.

Of particular interest with respect to the present investigation was the observation that metabolic parameters of cartilage activity were not increased in parallel with the development of degenerative joint disease. On the contrary, at 9 weeks, when significant pathological changes were present, all isotope label incorporations were diminished. At 12 weeks, the time of maximal pathology in this experimental model, thymidine, glycine, and sulphate incorporations were still low, although some increase toward levels in normal control animals was noted. These findings are in accord with the observations of Maroudas[7] and of Lust *et al.*[4] but contrast with reports of other investigations which described increased thymidine[5] and sulphate[5 6 10] incorporation in parallel with degenerative changes.

Differences observed in the above studies may be related to a number of factors, including whether human or animal tissues were studied, differences between animal species, differences between cartilage from different joints being analysed, analysis of cartilage from different depths of tissue,[7] duration and severity of disease, and experimental methodologies used. Organ culture studies in this investigation were performed by a modification of the methodology described by McKenzie *et al.*[17] designed to minimise the effect of cartilage 'shock' with stabilisation of tissue in culture. Their findings that $^{35}SO_4$

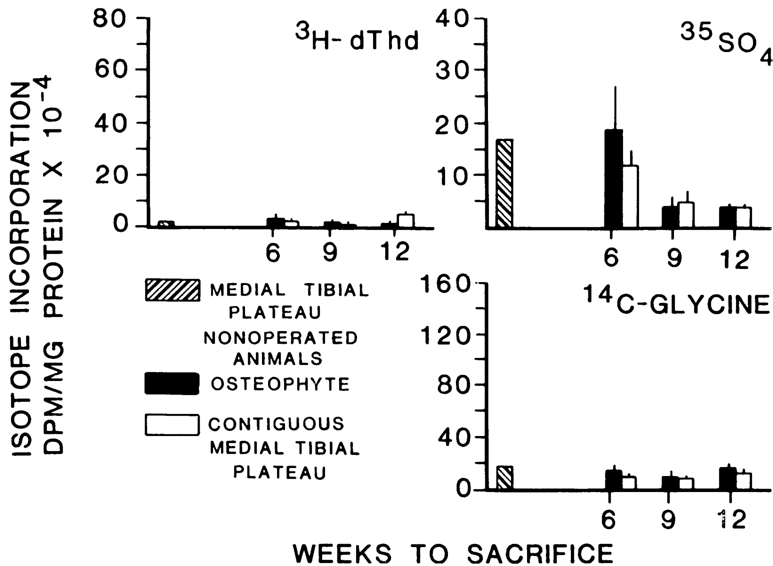

Fig. 4 *Incorporation of ^{3}H-thymidine (dThd), $^{35}SO_4$, and ^{14}C-glycine into osteophytes is compared with isotope incorporation into the contiguous medial tibial plateau of the same animals and into medial tibial plateaus of nonoperated control animals.*

incorporation increased in linear fashion with time accentuates the significance of the observed diminished isotope incorporation in our study. Comparison of results previously reported by us using qualitative autoradiographic techniques in the same experimental model[8] is of further interest in regard to differences in methodology. Those studies revealed increases in protein and glycosaminoglycan synthesis and in cell replication. The increased isotope incorporation in protein and glycosaminoglycan components of cartilage closely paralleled cartilage degenerative changes. Cell replication was increased early in cartilage, but was later diminished to control levels despite progression of degeneration. Sham-operated animals were unfortunately not included. Accordingly in that study metabolic changes resulting from arthrotomy could not be separated from those due to degenerative joint disease. Nevertheless, metabolic parameters were increased in contrast with the decreased or normal incorporations noted in the present study. Differences in results in the 2 studies using the same model may reflect variations in measurement of cartilage metabolism related to the qualitative autoradiographic technique versus direct quantitative measurements of isotope incorporation.

Differences in observations by various investigators could also be related to the manner in which observed isotope incorporations were expressed. Specifically, prior investigations have variably expressed isotope incorporation as a function of wet[4 7] or dry[2 3 5] weights of cartilage, uronic acid content,[1 9] or tissue DNA content.[5 6 10] For example, Thompson and Oegema[10] noted no correlation between sulphate incorporation and histological severity of osteoarthritis when incorporation was expressed as dpm per mg dry weight of tissue. When incorporation was expressed as dpm per microgram of DNA, however, statistically significant correlations with histological grade were noted. Incorporation expressed as dpm per mg protein was not assessed. Observations in the partial meniscectomy model used in the present investigation[12] have shown no apparent differences in cell to matrix ratios nor a change in cell number except for an increased cellular proliferation just immediate to localised areas of ulceration when normal rabbit knees are compared to those with osteoarthritic change. The increased DNA/protein ratios seen in femoral as compared with tibial cartilage in our study might well explain the increased isotope incorporations seen in femoral as opposed to tibial cartilage. Similar increases in incorporation noted in lateral as opposed to medial tibial plateau cartilage are not, however, explicable on the basis of differences in DNA/protein ratios. DNA/protein ratios in corresponding quadrants of normal and osteoarthritic cartilage were similar. Accordingly it seems unlikely that the interpretations of data comparing normal and partial meniscectomy animals would have varied significantly if other denominators had been used to express incorporation rates.

It might be speculated that in the present study errors in tissue sampling, whereby osteoarthritic

metabolic changes were diluted out by inclusion of degenerative lesions and normal tissue in the same sample, may have led to spurious results. To avoid such error analyses were performed on medial and lateral femoral and tibial condyles/plateaus separately, so that metabolic activities could be related to areas of maximal pathology. Findings in the medial femoral condyle, the site of maximal disease, did not differ significantly from results observed in the lateral femoral condyle of the same knee, nor from medial femoral condyle findings in sham-operated knees. Furthermore, although ulcerative erosions were seen primarily in a focal distribution at the weight-bearing areas of the medial femoral condyle, pitting lesions were diffusely distributed throughout the same condyles, showing the widespread nature of the lesion. Finally, studies in this same model[18] and in an experimental dog model of osteoarthritis[19] have demonstrated qualitative changes in proteoglycan composition in *all* areas of the operated osteoarthritic joint whether or not pathological changes could be demonstrated.

The lack of increased incorporation of ^{3}H-thymidine into osteophyte tissue was unexpected in view of the proliferative nature of the lesion found on histological study. This finding is particularly surprising in view of the high DNA/protein ratios observed in osteophytic tissue, in which case increased ^{3}H-thymidine incorporation would have been expected in parallel with increased tissue cellularity. Autoradiographic studies in this same model showed increased ^{3}H-thymidine incorporation into osteophytes at 2 and 5 weeks after partial-meniscectomy; at 8 and 12 weeks labelling of cells was similar to control findings.[8] The failure to observe increased ^{3}H-thymidine incorporation by osteophytes in the present study suggests that maximal osteophyte proliferation took place prior to the appearance of fully formed osteophytes at 6 weeks and later. Evidence of increased incorporation at 2 and 5 weeks, but not at 8 and 12 weeks, in the autoradiographic study is consonant with this hypothesis. Insensitivity of the isotope technique to measure slow rates of cell proliferation over the 20 h period of isotope exposure seems a less likely explanation, although not excluded in contributing to the findings. The decreased $^{35}SO_4$ incorporation is less surprising, in that these spurs characteristically show minimal stain with safranin-O,[12] consistent with decreased proteoglycan content.

Several hypotheses may be considered to explain the findings in the nonoperated left knees of the partial meniscectomy and sham-operated animals, wherein similar and at times identical responses to the operated knees were noted. The left knees of operated animals are not true controls, in that increased weight-bearing on the left knees probably occurs for some time after right knee surgery. Accordingly some of the responses observed might be related to compensatory increases in left knee stress. Left knee alterations in rabbits in which the right knee had been subjected to arthrotomy were reported by Rosenthal.[20] A transient rise in activity of synovial lysosomal enzymes was seen not only in the right knees, but also in the unoperated left knees of all animals studied. A review of the data provided in studies of osteoarthritis induced in dogs following section of the anterior cruciate ligament[19] reveals that the cartilage of control nonoperated knees showed increases in water content, increased ease of extractability of proteoglycan, and increased glactosamine/glucosamine ratios. Although these changes were less in degree than seen in the operated osteoarthritic right knee, the increases appeared significant. Floman *et al.*[21] noted differences in collagen metabolism between left knee control joints of meniscectomised animals and left knee control joints of the sham-operated animals, with increased ^{3}H-proline incorporation in the former. They suggested that the effect could be due to a humoral factor or occurred because the meniscectomised animals limped and increased the load on their control knees. Finally, it is possible that the metabolic responses observed represent a nonspecific response to anaesthesia and general stress rather than a response to the knee surgery per se. Control studies using normal unoperated animals undergoing anaesthesia, or analysis of joints other than the knee in partial meniscectomy animals, would be necessary to clarify this possibility.

The present studies of cartilage metabolism which used measurement of isotope incorporation appear to provide only partial information in the delineation of cartilage response to pathological change. Initial studies to investigate qualitative differences in cartilage metabolism between partial meniscectomy, sham-operated, and normal non-operated knees in the present investigation showed no obvious differences in partitioning of sulphated glycosaminoglycans between tissue and media. The reduced glycine incorporation observed at 3 weeks in both partial meniscectomy and sham-operated animals is unexplained. These overall findings suggest that no difference in cellular secretion of formed glycosaminoglycans exists between normal and osteoarthritic cartilage. Further studies to define qualitative differences in proteoglycan subunit synthesis and formation of proteoglycan aggregate at serial time points and at various intervals of disease severity are in progress.

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