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EXPERIMENTAL INVESTIGATION

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Human meniscal proteoglycan metabolism in long-term tissue culture

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Abstract For the purpose of human meniscal allografting, menisci have been maintained viable in in vitro culture. The influence of long-term tissue culture on the extracellular matrix metabolism of the meniscus has been studied. Fetal calf serum (FCS) was used as a supplement for the growth factors necessary to maintain optimal meniscal cell metabolism. A series of semilunar cartilage samples was cultured under serum-free conditions since foreign proteins could be responsible for immunological problems after eventual allografting. The proteoglycan metabolism in human menisci cultured in FCS-supplemented and in serum-free culture media was compared. To rule out any influence of topographical variations in glycosaminoglycan (GAG) content on proteoglycan (PG) metabolism, GAG concentrations within the tissue were determined, and sulphate (^{35}S) incorporation was studied in tissue samples with a comparable biochemical composition. Sulphate incorporation was preserved when 20% FCS was added to the nutrient medium. The meniscal tissue fibroblasts continued to produce ^{35}S-PG during 4 weeks of culture. The PG molecules were shown to consist of PG-aggregates, monomers and a low molecular-weight PG population. Newly synthesized GAG consisted of approximately 55% chondroitin 4- and 6-sulphate and 33% dermatan sulphate. In the presence of serum, ^{35}S incorporation in PG and in the PG-aggregate fraction significantly increased during the first 2 weeks and then decreased during the following 2 weeks of in vitro culture. Newly synthesized PG-aggregates were almost entirely accumulated in the tissue during these weeks. In the 3rd week the values for this parameter decreased slightly. ^{35}S-PG synthesis dramatically declined after 4 weeks of in vitro culture. Catabolism probably resulted in increased proportions of ^{35}S-PG in the incubation media. In the absence of serum, ^{35}S-PG production also increased in the 2nd week of culture. However, ^{35}S activity was almost exclusively found in small PG, and this material apparently diffused to the incubation media. Consequently, catabolism is higher, and the immobilization of ^{35}S-PG is poor when FCS is not added to the culture media. Our findings suggest that menisci are maintained in viable condition and may serve for allografting at least during 2 weeks of tissue culture.

Key words Meniscus culture · Transplantation · Proteoglycan

Introduction

With cryopreservation, biosynthetic activities in semilunar cartilage are diminished to less than 50% of normal control values, and only 10% of the meniscal cells show metabolic activity [3]. According to some authors, the fine architecture of γ-sterilized, lyophilized meniscal allografts is completely disrupted, and the tissue is non-viable [13].

Canine meniscal allografts implanted after 2 to 3 weeks of tissue culture survive 1 month in vivo [5]. In view of these results and of the successful use of fresh allografts for transplantation [21], we have opted for in vitro culture of semilunar cartilage. This technique would provide sufficient time for bacteriological and biochemical studies and for preparation of the recipient. The composition of the intracellular matrix of a tissue has an important influence on its properties [8]. Consequently, changes in the biochemistry of the extracellular matrix should be evaluated when this tissue is stored in vitro [19]. During storage, it is of paramount importance that the cells maintain their ability to function.

For the purpose of human meniscal allografting, menisci have been preserved using in vitro culture. The influence of long-term tissue culture on viability of the meniscus has been studied. To evaluate meniscal tissue metabolism during culture, we studied proteoglycan (PG) synthesis and the variations of glycosaminoglycan (GAG) concentrations in this tissue. Meniscal samples were cultured in semisynthetic nutrient media with and without 20% fetal calf serum (FCS). Human articular cartilage samples were maintained in tissue culture for up to 12 weeks using this protocol [16]. GAG and collagen chemistry have been reported to vary according to the site of harvesting [1, 2, 6]. To rule out any influence of topographical variation on the interpretation of the results, meniscal tissue samples with a comparable biochemical composition at the start were selected.

Materials and methods

Tissue samples

Tissue samples were obtained from cadaveric knee joints within 12 h of death. Seven meniscal samples were obtained from three different donors (Table 1). The first two menisci (nos. 1 and 2) were removed from the left knee of a 34-year-old man, who had died in a motor vehicle accident. Four other menisci (nos. 3–6) were obtained from a 65-year-old man, who had died of gastric carcinoma. The seventh meniscus was harvested from a 41-year-old woman; the cause of death was a cerebrovascular accident. All menisci were macroscopically intact.

Distribution of GAG content

Two menisci (nos. 1 and 2) were kept in culture in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% FCS for 2 weeks before experimental use. After this culture period, the menisci were cut to eight full-thickness samples as shown in Fig. 1. Both the anterior and posterior horns had previously been shown to have different tissue GAG levels [1]. These parts of the menisci were not used for the experiments.

Table 1 Origin and use of meniscus samples

No.	Sex	Age (years)	Location	Culture period before start of experiments
1	M	34	Lateral left	2 weeks
2			Medial left	2 weeks
3	M	65	Lateral left	Immediately
4			Medial left	Immediately
5			Medial right	Immediately
6			Lateral right	Immediately
7	F	41	Lateral left	Immediately

The dry weight of the samples was measured following lyophilization overnight [14]. Subsequently, the samples were allowed to swell again in 0.067 *M* phosphate, pH 6.4, and were digested at 65° C for 2 h with papain (0.6 mg/ml in 0.067 *M* phosphate, pH 6.4, containing 2 m*M* *N*-acetyl-cysteine and 2 m*M* ethylene diamine tetra-acetic acid, EDTA). An aliquot was then used to assay the total GAG content using the dimethylmethylene blue assay [7] and chondroitin sulphate as a standard. The values were expressed per milligram dry weight meniscal tissue [16].

Long-term tissue culture

Menisci nos. 3–7 were cut to full-depth samples of 2 × 3 × 5 mm and cultured for 4 weeks. The site of harvesting had been determined by the results of previous analyses of GAG content in the tissue. The samples were incubated with DMEM supplemented with antibiotics (penicillin 10 U/ml, streptomycin 10 μg/ml, fungizone 0.025 μg/ml) and L-glutamine (0.002 *M*/ml). Medium was replaced twice a week. Menisci nos. 3–6 were incubated with DMEM supplemented with 20% FCS during the entire period of culture. Meniscus no. 7 was incubated in serum-free medium, FCS being added to the medium only during the ^{35}S-labelling week.

^{35}S-labelling protocol

Four tissue samples per meniscal body from menisci nos. 3–7 were cultured for 4 weeks. $Na_2{}^{35}SO_3$ (5 μCi/ml) was used as a radioactive precursor of PG during 1-week periods. For each next piece of the same meniscus, ^{35}S-labelling was started 1 week later than for the previous one. With this procedure the effects of long-term meniscal tissue culture on PG metabolism during four 1-week ^{35}S-labelling periods could be evaluated. ^{35}S-incorporation rates in tissue extracts, tissue residues from the extraction, and media allowed us to evaluate PG synthesis rates/mg dry weight during these 1-week periods [16]. From the comparison of the amount of ^{35}S in media and in tissue extracts plus residues, conclusions could be drawn about the diffusion rates of the respective PG pools from the culture tissue. The escape of PG from culture cartilage might reflect catabolic events [11]. The same data were used to compute PG extractability. From this extractability conclusions could be drawn about the integrity of the meniscal matrix.

Extraction of extracellular matrix PG

After the radiolabelling periods all samples were removed, lyophilized in order to obtain their dry weights, and allowed to

swell again in 4.0 *M* guanidinium chloride in 0.05 *M* Na·acetate, pH 5.8, with protease inhibitors [12]. Dissociative extraction took place in this solution for 1 week. Before extraction the meniscal pieces were pulverized in a stainless steel mortar. On the 5th day of the extraction period, the remaining tissue was cut into smaller pieces and extracted for another 2 days. This procedure was found to produce higher PG extraction rates. The extract was dialysed twice for 2 days against 0.4 *M* guanidinium chloride in 0.05 *M* Na·acetate, pH 5.8. Aliquots of the retentates were used to assay ^{35}S activity and GAG content and were eluted on Sepharose 2B gels.

GAG and ^{35}S-PG content

Aliquots of the retentates of extracts and of the media were further used to assay ^{35}S activity and GAG content. The dimethylmethylene blue assay was used [7], and chondroitin sulphate served as a standard. Tissue residues remaining after extraction were washed in 0.067 *M* phosphate, pH 6.4, to remove the unincorporated label and digested at 65° C for 2 h with papain (0.6 mg/ml) in 0.067 *M* phosphate, pH 6.4, containing 2 m*M* acetylcysteine and 2 m*M* EDTA. An aliquot was then used to assay non-extractable total GAG and ^{35}S-labelled PG. Total GAG in extractable and non-extractable pools and in culture media were summed to obtain total values for this item expressed per milligram dry weight of tissue. Calculations of ^{35}-S incorporation in tissue were based on values obtained from the sum of ^{35}S-PG in extract and residue. In this way any influence of PG extractability on this parameter was ruled out.

Sepharose 2B chromatography

Two milliliters of each retentate were applied on Sepharose 2B and eluted with 0.067 *M* phosphate, pH 6.8. The eluted fractions were counted for radioactivity. Radioactive material eluted in three fractions, which had previously been identified as ^{35}S-PG-aggregates, ^{35}S-PG-monomers and a third fraction containing smaller PG fragments [9, 11, 12, 15, 18]. Total amounts of PG-aggregates, monomers and small PG were defined as total ^{35}S activity eluted in the three pools.

Relative amounts of aggregates (or monomers or small PG) in the PG pool were defined as ^{35}S activity of pool I (or pool II or pool III) × 100/(^{35}S activity of pool I + II + III). They gave an indication of the ultimate quality of the PG molecule and possible information on catabolic events in the meniscus.

From radioactivity measurements on column effluents of a known volume of the retentate, the incorporation rate of radiosulphate in extractable PG (aggregates, monomers and small PG) per milligram of dry weight was calculated. Incubation media of these tissue cultures were also dialysed against 0.4 *M* guanidinium chloride in 0.05 *M* Na·acetate, pH 5.8, and the same investigations were performed as on the retentates of the tissue extracts. In this way identical variables could be calculated: total amounts of ^{35}S-PG-aggregates, monomers and small PG liberated in the media per milligram of dry weight meniscus in culture per week.

Characterization of GAG in ^{35}S-PG

Extracts from tissue samples after the 1st week of culture in the presence of serum were used to characterize ^{35}S-GAG in newly synthesized PG. The different GAG were characterized after digestion of ^{35}S-PG with specific chondroitinases [20]. The resulting disaccharides were separated from undigested PG by gel permeation chromatography on Sephadex G50 gels in 0.067 *M* phosphate, pH 6.8.

Statistics

Where appropriate, mean values, standard deviation (SD) and coefficient of variation (CV = 1 SD × 100/mean) were calculated.

Results

Distribution of GAG in the meniscus

Eight samples per meniscus (nos. 1 and 2) were studied for their total GAG content (Fig. 1). Mean values for total GAG were 21.3 and 20.0 µg/mg dry weight in the tissue samples of the two menisci. Lower values for total GAG (15.0 and 12.0 µg/mg dry weight) were found in the internal fragments of the respective menisci.

Total GAG content of long-term cultured menisci

GAG levels in tissue and media are shown in Table 2. Tissue GAG levels were lower in the samples cultured in media supplemented with FCS than in samples cultured un-

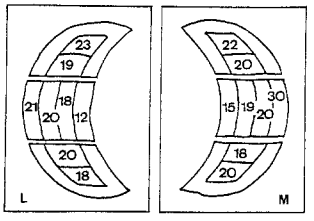

Fig. 1 Glycosaminoglycans (GAG) in human menisci: distribution of GAG content of two menisci (nos. 1 and 2) obtained from the same donor. Values are expressed as µg GAG/mg dry weight

Table 2 Total amount of GAG in tissue extract and residue and of GAG released into culture medium during 4 weeks of culture. Tissues were cultured in DMEM continuously *(C)* supplemented with 20% FCS (menisci nos. 3–6) or in DMEM without *(W)* 20% FCS in the prelabelling period (meniscus no. 7). GAG values are expressed as µg/mg dry weight of tissue. *GAG release/week* GAG lost from the tissue samples per week

	Week 1		Week 2		Week 3		Week 4	
	C	W	C	W	C	W	C	W
GAG extract (E)	6.3 (14.9)	13.8	4.9 (24.3)	9.3	4.0 (50.4)	11.2	5.0 (20.8)	11.6
GAG residue (R)	1.5 (24.0)	3.2	0.8 (26.0)	1.8	0.9 (33.0)	1.3	1.3 (29.7)	1.3
GAG tissue (E + R)	7.8 (14.7)	17.0	5.7 (17.3)	11.0	4.9 (37.1)	12.5	6.3 (0.1)	12.9
GAG medium (M)	21.7 (14.0)	10.8	33.2 (9.9)	21.1	51.1 (20.9)	35.1	57.9 (14.4)	51.8
Total GAG (E + R + M)	29.5 (11.8)	27.8	39.8 (6.0)	32.1	56.0 (16.4)	47.6	64.2 (12.2)	64.7

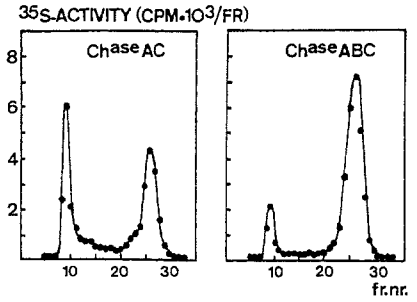

Fig. 2 Sephadex G50 chromatography of chondroitinase AC *(left)* and chondroitinase ABC *(right)* ^{35}S-PG digests. *First fractions* undigested material, *second fraction* chondroitinase-digested material, *ordinate* ^{35}S-radioactivity in cpm/fraction, *abscissa* eluted fraction numbers

der serum-free conditions. However, these tissue specimens were obtained from different donors. Despite the important losses of GAG to the nutrient media, tissue GAG concentrations remained relatively constant during subsequent weeks of culture in media with and without FCS. During the 4 weeks of culture, there was a tendency to a decreased loss of GAG from the tissues grown in the presence of serum. The opposite was true for tissues grown under serum-free conditions. Total GAG levels per milligram dry weight of tissue were derived from the calculated sums of the values found in tissue and culture media (cumulative values) over subsequent weeks. These total GAG levels continuously increased during subsequent 1-week periods in serum-free media and in nutrient media supplemented with FCS. The bulk of GAG were recovered from the nutrient media.

Characterization of ^{35}S-GAG in meniscal PG

Representative Sephadex G50 chromatography curves of chondroitinase AB/ABC ^{35}S-PG digests are shown in Fig. 2. After digestion with chrondroitinase AC, approximately 55% of the GAG were identified as chondroitin 4- and 6-sulphate. The experiments in which chondroitinase ABC was used showed that 88% of the GAG was digested. Consequently, chondroitin 4- and 6-sulphate plus dermatan sulphate accounted for 88% of the material (12% remained undigested). It may be concluded that 33% of the GAG consisted of dermatan sulphate.

^{35}S-incorporation in PG of tissue and incubation media

Total amounts of both ^{35}S incorporated in matrix PG per milligram dry weight tissue and ^{35}S-labelled PG released into the medium per milligram dry weight per week were calculated from the areas under the Sepharose Cl 2B chromatography curves and are shown in Table 3.

Except for the 4th week of culture, the total PG synthesis rates were approximately 10 times higher in the menisci cultured in media supplemented with FCS than in those cultured under serum-free conditions. This may reflect either the dependence of PG synthesis on growth factors or the different source of the samples.

^{35}S incorporation in PG of tissue and media (CPM Tot./mg) significantly increased during the 2nd week of culture. This increase was more pronounced in tissues cultured under serum-free conditions (× 5.7) as compared with tissues cultured in the presence of FCS (× 2.6).

Total ^{35}S incorporation then declined during the 3rd and 4th week of culture in tissues cultured in the presence of serum. ^{35}S-PG synthesis remained high in the samples cultured under serum-free conditions. However, most of the ^{35}S activity did not accumulate in the tissue.

Losses of newly synthesized PG from the tissues were reflected by the tissue/medium ^{35}S-PG ratios which, in the FCS experiments, increased during the 2nd week but constantly decreased during the last 2 weeks. In the serum-free experiment the tissue/medium ^{35}S-PG ratio continued to decrease from the 1st week of culture.

Table 3 ^{35}S incorporation in meniscus samples cultured in a medium continuously *(C)* supplemented with 20% FCS (menisci nos. 3–6) and in samples cultured in a medium *(W)* without 20% FCS in the prelabelling period (meniscus no. 7). ^{35}S activities are expressed as cpm/mg dry weight tissue/week. Mean values and coefficients of variation (1 SD × 100/mean) are given for the experiment in media supplemented with FCS. One tissue sample was used for the experiment under serum-free conditions. (*ND* experiment not done, *CPM Extr./mg* and *CPM Res./mg* ^{35}S activity in 4 *M* guanidinium chloride-extractable and in non-extractable (tissue residue) matrix molecules, *CPM Med./mg* ^{35}S activity in the incubation media, *CPM Tot./mg* cumulative ^{35}S activity in proteoglycan recovered from extract, residue and media, ^{35}S T/M ^{35}S tissue/medium ratios)

	Week 1		Week 2		Week 3		Week 4	
	C	W	C	W	C	W	C	W
CPM Extr./mg	4759 (32)	404	14339 (27)	1607	6131 (14)	ND	1324 (33)	236
CPM Res./mg	520 (74)	87	2210 (12)	177	676 (25)	ND	250 (23)	13
CPM Med./mg	4109 (28)	200	8217 (55)	2139	4501 (48)	ND	1985 (26)	3465
CPM Tot./mg	9389 (27)	691	24708 (28)	3923	11309 (23)	ND	3559 (20)	3716
^{35}S T/M	1.29/1	2.46/1	2.01/1	0.50/1	1.51/1	ND	0.79/1	0.07/1

Table 4 Tissue samples cultured in media continuously *(C)* supplemented with 20% FCS and tissue samples cultured in serum-free media *(W)* in the prelabelling period. Mean values and coefficients of variation are given (1 SD × 100/mean) [*ND* experiment not done, *% ^{35}S-Aggr, % ^{35}S-Mono* and *% ^{35}S small PG* percentage values of ^{35}S activity in ^{35}S-PG aggregates, ^{35}S-PG monomers and small PG, *CPM ^{35}S-Aggr, CPM ^{35}S-Mono* and *CPM ^{35}S small PG* ^{35}S activity incorporated in ^{35}S-PG aggregates, monomers and small PG of meniscus samples (mg^{-1} dry weight · week^{-1}) *% Aggr. tiss, % Mono tiss* and *% small PG tiss* tissue and medium values of ^{35}S activity of the respective pools (aggregates, monomers and small PG) were summed up, and their distribution coefficients between tissue and media were computed]

	Week 1		Week 2		Week 3		Week 4	
	C	W	C	W	C	W	C	W
% ^{35}S-Aggr.	21.3 (10)	19.0	17.3 (23)	10.0	17.3 (36)	ND	19.0 (18)	2.0
% ^{35}S-Mono.	34.3 (16)	38.0	39.7 (11)	27.0	40.8 (17)	ND	39.5 (15)	2.0
% ^{35}S small PG	44.5 (16)	43.0	43.0 (17)	63.0	42.0 (32)	ND	41.8 (17)	96.0
CPM ^{35}S-Aggr.	1134.0 (30)	76.8	2900.0 (36)	161.0	1204.0 (45)	ND	308.0 (42)	4.7
CPM ^{35}S-Mono.	1858.0 (41)	154.0	6692.0 (34)	43.0	2814.0 (28)	ND	621.0 (29)	4.7
CPM ^{35}S small PG	2288.0 (14)	174.0	6959.0 (16)	1012.0	2784.0 (25)	ND	649.0 (29)	226.6
Medium								
% ^{35}S-Aggr.	0.8 (100)	9.0	2.3 (100)	1.0	11.0 (90)	ND	2.3 (82)	3.0
% ^{35}S-Mono.	7.0 (108)	26.0	23.7 (43)	3.0	25.3 (48)	ND	15.3 (42)	1.0
% ^{35}S small PG	92.3 (7)	65.0	74.0 (13)	96.0	63.7 (20)	ND	82.5 (8)	96.0
CPM ^{35}S-Aggr.	38.0 (140)	18.0	185.0 (100)	21.4	523.0 (60)	ND	50.0 (99)	104.0
CPM ^{35}S-Mono.	294.0 (110)	52.0	1650.0 (20)	64.2	1411.0 (100)	ND	306.0 (50)	35.0
CPM ^{35}S small PG	3777.0 (30)	130.0	6382.0 (70)	2053.0	2806.0 (30)	ND	1630.0 (30)	3326.0
Accumulation								
% Aggr. tiss.	96.8	81.0	94.0	88.3	69.7	ND	86.0	4.3
% Mono. tiss.	86.3	74.8	80.2	40.1	66.6	ND	67.0	11.9
% small PG. tiss.	37.7	57.2	52.2	33.0	49.8	ND	28.5	6.4

Tissue-extracted ^{35}S-PG increased approximately 3 times for the menisci continuously supplemented with FCS and approximately 4 times for meniscus no. 7, which was supplemented with FCS only during the labelling period. However, newly synthesized PG did not properly accumulate in the tissue when the samples were cultured under serum-free conditions. For these meniscal samples, the increase in ^{35}S-PG content was higher in the medium (× 10) than in the tissue (× 3.5). In the samples continuously cultured in DMEM supplemented with FCS, the increase in ^{35}S-PG content was higher in the tissue (× 3) than in the corresponding media (× 2). Tissue ^{35}S-PG levels fell under the baseline values during the 4th week of culture in menisci cultured with or without FCS.

Qualitative analysis of newly synthesized ^{35}S-PG

Aliquots of extracted material and of dialysed nutrient media were passed through Sepharose 2B gels to separate and calculate relative proportions of PG aggregates, monomers and small PG. These values were analysed in the 1st, 2nd, 3rd and 4th weeks of the ^{35}S-labelling period. The results are presented in Table 4. Sepharose Cl 2B elution patterns of tissue extracts and incubation media from the samples of one meniscus are shown in Fig. 3. They illustrate the changes in synthesis and physicochemistry of ^{35}S-PG during the 4 weeks of culture.

Relative amounts of different PG populations

For menisci cultured in media supplemented with FCS (nos. 3–6), relative amounts of aggregates, monomers and small PG did not significantly change in the tissue during the 4 weeks of meniscal culture. The relative amounts of both aggregates and monomers were much lower in the medium than in the tissue. Mainly small PG were found in these incubation media.

In the menisci that had been cultured under serum-free conditions, the relative amounts of aggregates and monomers were significantly lower than in the specimens cultured in FCS. Furthermore, these values significantly decreased in tissue and in medium during the consecutive labelling periods. The ^{35}S-PG aggregates and monomers almost disappeared in the 4th week of culture. The relative amount of small PG increased.

Absolute amounts of different PG populations

From the values of ^{35}S-activity · mg^{-1} · week^{-1} in radiolabelled material (Table 3) and the proportional values of the PG subpopulations (Table 4), we computed absolute amounts of ^{35}S activity found in PG-aggregates, monomers and small PG in tissue and medium, respectively. Summation of tissue and medium ^{35}S-PG first gave us an idea of total metabolic activity of the cultured samples

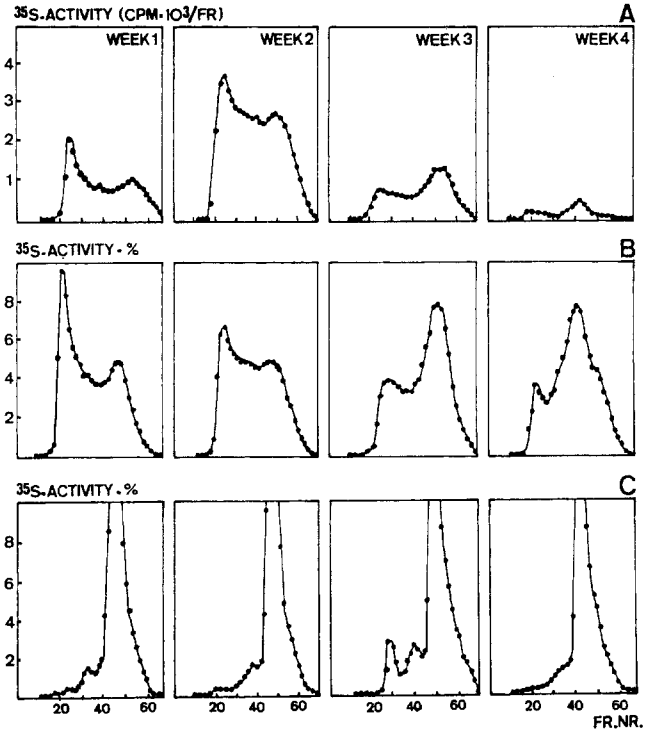

Fig. 3 Sepharose Cl 2B chromatography curves of ^{35}S-PG: **A** tissue extracts: absolute amounts of ^{35}S radioactivity in PG illustrate changes in PG synthesis levels per week; **B** tissue extracts: chromatograms in **A** are now represented with identical areas under the curves to illustrate changes in proportions of PG subgroups during the 4-week culture period; **C** incubation media: relative amounts of ^{35}S radioactivity in media PG (graphs are represented with identical areas under the curves) illustrate changes in proportions of PG subgroups released in the media during the 4-week culture period. *Ordinate* Absolute (**A**) and relative (**B** and **C**) amounts of radioactivty; *abscissa* eluted fraction numbers

(Table 3, CPM Tot./mg). Absolute amounts of different PG retained in the tissues are given in Table 4 (CPM ^{35}S-aggregates, monomers and small PG). ^{35}S-PG-aggregates were almost entirely retained in the extracellular matrix during the 1st week of culture (approx. 97%) when tissue samples were cultured in the presence of serum. Retention of PG-aggregates decreased somewhat from the third week on. Meniscal tissues in serum-free media retained lower amounts of ^{35}S-PG aggregates than did tissue samples cultured with FCS. Virtually no ^{35}S-PG-aggregates were found in the tissues maintained in serum-free culture media during 4 weeks.

Discussion

The anatomical site in the meniscus from which tissue samples were obtained for long-term tissue culture experiments was determined by experimental results of GAG distribution in the meniscus. Compared with other sites in the meniscus, the internal meniscal margin contained significantly lower GAG amounts. GAG amounts are lower in the meniscal horns than in the rest of the meniscal body [1]. To rule out any effect of the insertion of the meniscus on the synovial membrane, the external margins were also excluded. Therefore, only fragments from a median longitudinal slice were used after removal of the horns. The GAG concentration in these tissue samples ranged between 18 and 30 µg/mg dry weight. These results are in agreement with values reported elsewhere for non-degenerative menisci [10].

Normal human meniscal PG contain approx. 40% chondroitin 4-sulphate, 10%–20% chondroitin 6-sulphate, 20%–30% dermatan sulphate and 15% keratan sulphate [10]. These parameters were studied in the cultured human menisci. The meniscal fibrochondroblasts synthesized PG with GAG proportions comparable to those reported in a normal extracellular matrix.

The periodical ^{35}S-labelling protocol allowed us to study PG metabolism in cultured human meniscal tissue. Part of the ^{35}S-labelled PG was lost from the tissue and diffused into the incubation medium, the remainder being immobilized in the intracellular matrix. Summation of tissue and medium ^{35}S activity provided a variable for the synthetic function of the meniscal cells per week. The ratio of ^{35}S-PG in matrix/medium reflected the loss of newly synthesized PG from the intracellular matrix and was used as a measure of catabolic activity within the tissue.

The ^{35}S incorporation rates in the 1st week of culture may reflect in vivo metabolism. The menisci continued to produce ^{35}S-PG during the 4 weeks of culture. It can thus be concluded that the meniscal tissue cells remain metabolically active and viable. However, the meniscal samples obtained from two different donors had widely different ^{35}S-PG synthesis rates. In the 1st week of culture, the experimental conditions were identical for both tissue samples in that FCS was present in the nutrient medium. Similar divergent ^{35}S incorporation rates were also observed in human articular cartilage in vitro [4]. Because of their high molecular weight, most of the ^{35}S-PG-aggregates and large amounts of the monomeric ^{35}S-PG accumulated in the tissues. The small PG predominantly diffused into the incubation media.

^{35}S incorporation in PG in meniscal tissue samples cultured long term followed the same kinetics as previously described for human articular cartilage cultured long term [11, 16]. In the presence of serum, ^{35}S incorporation in PG significantly increased during the first 2 weeks and then decreased during the following 2 weeks of in vitro culture. The retention of ^{35}S-PG in the tissues (^{35}S tissue/medium ratios) reached an optimum after the first 2 weeks of incubation. Approximately two-thirds of the newly synthesized PG were then recovered from the tissues.

^{35}S activity incorporated in tissue aggregates doubled during the first 2 weeks. Newly synthesized PG-aggregates had almost entirely accumulated in the tissue during

this time. In the 3rd week the values for this parameter decreased slightly. Moreover, an increase of relative amounts of PG-aggregates was observed in the media during the 3rd week of culture in the presence of serum. This indicates that higher molecular weight PG (aggregates) are also able to escape from the tissue at that point in the experiment. ^{35}S-PG synthesis dramatically declined after 4 weeks of in vitro culture. Catabolism could have resulted in increased proportions of ^{35}S-PG in the incubation media.

The effects of the serum-free experiment could only be studied during the 2nd and following weeks of culture. ^{35}S-PG production increased in the 2nd week, regardless of the presence of FCS in the culture media. Under serum-free conditions ^{35}S activity was almost exclusively found in small PG, and this material apparently diffused into the incubation media. Consequently, catabolism is higher and the immobilization of ^{35}S-PG is poor when FCS is not added to the culture media.

All menisci showed the highest amounts of tissue GAG in the 1st week of culture. These values represent the in vivo meniscal tissue GAG amounts and are consistent with those reported elsewhere in menisci with grade 3 to 5 degenerative changes [10]. Progressively increasing values were found for total GAG (tissue and culture media) during the 4 weeks of culture. The cumulative values of total GAG in the media were calculated to illustrate the total amount of GAG that escaped from the meniscal tissue during the culture period. It is assumed that loss of GAG is independent of the anatomical location in the meniscus so that the values found in the previous weeks can be considered representative of the whole meniscus during this time. Tissue GAG progressively escaped into the nutrient media.

As a general conclusion, it can be stated that PG metabolism and accumulation in human menisci remain adequate during the first 2 weeks of tissue culture when serum is present in the incubation media. These parameters reflect overall cellular function. If FCS is replaced by human adult serum, the culture conditions allow preservation of semilunar cartilage for transplantation purposes [17].

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