

# The influence of hydrocortisone on aggrecan metabolism in human articular chondrocyte cultures: Comparison of two different matrices

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## Abstract

### Objective

Numerous reports of negative effects as well as protective effects of glucocorticoids on articular cartilage convinced us to study the influence of hydrocortisone on aggrecan synthesis of isolated phenotypically stable human articular chondrocytes cultured in two different matrices.

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### Methods

Macroscopically normal human articular cartilage was obtained from femoral condyles within 24 hours postmortem. Chondrocytes were isolated and cultured in gelled agarose or in alginate. After 14 days in culture, hydrocortisone was added for 5 days at concentrations ranging from 0.005 µg to 1 mg/ml for the agarose cultures and from 0.005 µg to 1 µg/ml for the alginate culture system. Aggrecan synthesis was measured by the incorporation of <sup>35</sup>Sulphate, and the proportion of neosynthesized aggrecan that bound to hyaluronan to form aggrecan aggregates was analyzed by gel chromatography.

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### Results

At concentrations from 0.005 to 1 µg/ml, hydrocortisone was found to produce a similar dose-dependent stimulation of aggrecan synthesis in both matrices. The synthesis of aggrecans remained at the same level for concentrations of 1 µg/ml up to 100 µg/ml of hydrocortisone. When supraphysiological concentrations of hydrocortisone were added the aggrecan synthesis rate plateau declined. Simultaneously with the increase in aggrecan synthesis, the proportion of low-molecular weight <sup>35</sup>S-proteoglycans decreased in favour of <sup>35</sup>S-aggrecan aggregates and monomers in the agarose system. The chondrocytes cultured in alginate showed this increase of aggrecan aggregates and monomeric aggrecans in both the cell-associated and the inter-territorial matrix.

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### Conclusion

Hydrocortisone is a stimulator of aggrecan synthesis by normal human articular chondrocytes cultured in vitro. The two culture systems (agarose and alginate) tested in this experiment showed a comparable aggrecan synthesis rate, increasing under the influence of hydrocortisone at concentrations up to 1 µg/ml. The proportions of <sup>35</sup>Sulphate incorporated in aggrecan aggregates and monomeric aggrecan were also higher under the influence of hydrocortisone.

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### Key words

Chondrocytes, aggrecans, hydrocortisone, glucocorticoids, agarose, alginate.

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## Introduction

Glucocorticoids are an important therapeutic tool for the medical practitioner. They are administered systemically for their dramatic anti-inflammatory actions in chronic inflammatory joint disease. Intra-articular injections of corticosteroids are commonly used in the local treatment of inflammatory joint diseases. In osteoarthritis (OA), mainly in episodes of inflammatory exacerbations, the local administration of glucocorticoids is a general practice. Nevertheless, the use of corticosteroids in this indication remains controversial. Some authors reported deleterious effects on cartilage. Intra-articular injections of glucocorticoids into the joints of laboratory animals have been shown to result in severe degenerative cartilage changes (1-6). *In vivo*, hydrocortisone has been reported to provoke a marked decrease in glycine utilization by rabbit articular chondrocytes, indicating a decline in the protein synthesis rate (7). Chondrocyte anabolism *in vitro*, including aggrecan and type II collagen synthesis, may be suppressed by glucocorticoids (8-10). A decrease in the <sup>35</sup>Sulphate-incorporation of human articular cartilage cultured *in vitro* has also been reported (11).

In spite of these reports of the detrimental action of glucocorticoids, there is evidence of chondroprotective effects of intra-articular corticoids in human OA (12) and in experimental models of OA, such as in the meniscectomized rabbit model (13,14), in the chemically induced cartilage damage in the guinea pig (15), and in the Pound-Nuki dog model of OA (16,17). In mice maintenance of the synthesis of large proteoglycans of articular cartilage by glucocorticoids has been described (18). The protective effect has been ascribed to the suppression of the active form of chondrocyte-derived metalloproteases (stromelysin) by glucocorticoids (12,17,19).

The present study was undertaken to investigate the direct effects of hydrocortisone on the aggrecan metabolism of isolated normal human articular chondrocytes cultured in gelled agarose or in alginate. Human chondrocytes and other mammalian cells are readily grown under monolayer conditions. However, chondrocytes cultured in this manner

rapidly dedifferentiate to fibroblasts. These dedifferentiated cells synthesize an extracellular matrix different from that found in human articular cartilage. Agarose and alginate are polysaccharides naturally derived from seaweed. They both mimic the extracellular matrix of articular cartilage. Agarose is a thermally reversible hydrogel that solidifies as a result of hydrogen-bound formation. Chondrocytes cultured in gelled agarose synthesize cartilage-specific collagens and extracellular matrix aggrecans, indicating that the cells maintain their phenotype (20, 21). This culture method was used because of the experience in our laboratory with this matrix and also because it allows exogenous factors access to the chondrocytes. Alginate is an ionic hydrogel that solidifies in the presence of divalent cations. This matrix also permits the chondrocytes to maintain their phenotype when cultured *in vitro*, synthesizing macromolecules such as cartilage-specific aggrecans and collagens, and to assemble them into a well-ordered matrix as present in the articular cartilage (22-24). The advantage of alginate is the reversibility of the gelled condition of this matrix, allowing the study of the cell-associated matrix (CAM) and of the interterritorial matrix (ITM) surrounding the chondrocytes. The intercellular matrix of cartilage is composed of two compartments (25): the CAM, subdivided into a thin pericellular matrix lying close to the chondrocyte, and adjacent to this a territorial matrix. The ITM forms the largest domain of the intercellular matrix.

Under these two culture conditions chondrocytes produce cartilage-specific aggrecans (AG). AG-aggregates and AG-monomers are immobilized in the artificial matrix, while smaller degradation products escape into the nutrient medium (21, 25, 26). The high reproducibility of the results renders the two models useful to study the influence of drugs on chondrocyte AG metabolism.

## Materials and methods

### Isolation of articular chondrocytes

Human articular chondrocytes were isolated as described elsewhere (27, 28), with a few modifications. Briefly, human articular cartilage was obtained at au-

topsy from femoral condyles of different donors within 24 hours postmortem. All donors had died after a short illness. None of them had received corticosteroids or cytostatic drugs. Visually intact cartilage was harvested and prepared for culture. Cartilage removed from the femoral condyles was diced into small fragments and the chondrocytes were isolated by sequential enzymatic digestion (hyaluronidase, pronase and collagenase) of the extracellular matrix as described in detail (29). Isolated cells were then centrifuged for 10 min. at 800 rpm, washed 3 times in DMEM with 10% FCS, tested for viability (Trypan Blue exclusion test) and counted. More than 95% of the cells were usually viable after isolation.

#### *Cultures in agarose*

Chondrocytes were cultured in gelled agarose as previously described (20), with some modifications (21, 29). The cultures were set up in 3.6 ml cryotubes (Nunc). The agarose used was ultra low-gelling temperature agarose (Type IX, gelling at  $< 15^{\circ}\text{C}$  and remelting at  $> 50^{\circ}\text{C}$ ; Sigma). 3% agarose in distilled water was autoclaved twice for 15 min. and stored at  $4-8^{\circ}\text{C}$  prior to use. The cryotubes were coated with 100  $\mu\text{l}$  of this 3% agarose solution and the coating was then allowed to gel at  $4-8^{\circ}\text{C}$ . Chondrocyte suspension cultures were established in 1.5% agarose in DMEM as previously reported (29). Coated cryotubes were filled with 300  $\mu\text{l}$  of chondrocyte/agarose suspension and kept at  $4-8^{\circ}\text{C}$  for 15 min. to allow the agarose to gel. The final cell density was approximately  $1.5 \times 10^6$  chondrocytes per culture. Three ml of the appropriate incubation medium were then added and the cultures were placed in an incubator at  $37^{\circ}\text{C}$  under 5%  $\text{CO}_2$  for 2 weeks prior to the start of the experiments, thereby allowing the cells to reach an equilibrium. Nutrient medium plus 50  $\mu\text{g}$  per ml of freshly dissolved ascorbate was replaced 3 times weekly. Three series of cultures were started with chondrocytes obtained from 5 different donors (donor 1-5).

#### *Cultures in alginate gel*

Chondrocyte cultures in alginate beads were prepared as described elsewhere

(22), with some modifications. Chondrocytes suspended in one volume of double-concentrated Hanks' balanced salts solution without calcium and magnesium (HBSS; Gibco) were carefully mixed with an equal volume of 2% alginate (low-viscosity alginate from *Macrocystis pyrifera*; Sigma) in HBSS, autoclaved for 15 min. The final cell concentration was  $5 \times 10^6$  chondrocytes per ml in 1% alginate. The chondrocyte/alginate suspension was then slowly dripped through a 23-gauge needle into a 102 mM calcium chloride solution. The beads were allowed to polymerise for 10 min at room temperature. The calcium chloride was then removed, the beads were washed 3 times with 0.15 M sodium chloride and finally cultured in 4 ml of DMEM with 10% FCS and 50  $\mu\text{g}$  ascorbate per ml. The nutrient medium was replaced 3 times weekly. The chondrocytes were cultured in a 6-well plate with  $1 \times 10^6$  cells per culture (each well containing 20 alginate beads;  $\pm 50,000$  chondrocytes per bead) for 14 days prior to the start of the experiments. Three series of cultures were started with chondrocytes from 3 different donors (donors 6-8).

#### *Quantitative determination of total $^{35}\text{S}$ -labelled proteoglycans synthesized in agarose cultures*

Each test consisted of a 5-day incubation period in the absence or presence of hydrocortisone. For the last 48 hours of the test period fresh medium including hydrocortisone and  $^{35}\text{S}$  sulphate was added. Hydrocortisone was used since it is the physiologically occurring glucocorticoid, and consisted of a solution of hydrocortisone sodium succinate in phosphate buffered saline with benzylic alcohol q.s. (Solucortef®; Upjohn). The contents of the vials were diluted to the desired concentrations of 0.005  $\mu\text{g}/\text{ml}$  to 1  $\text{mg}/\text{ml}$ .

$^{35}\text{S}$ -incorporation in aggrecan was investigated using  $\text{Na}_2^{35}\text{SO}_4$  as a radioactive precursor. Under most of the *in vitro* conditions tested by us so far, a large part of the  $^{35}\text{SO}_4$  used by phenotypically stable chondrocytes was incorporated in aggrecan. Considering the amount of pooled culture medium that ultimately was analysed after chromatography, the specific activity of the incubation medium, the

decay of  $^{35}\text{S}$  sulphate, the labelling period in hours and the numbers of cells per culture, sulphate incorporation was expressed as pg  $\text{SO}_4$  incorporated per  $1 \times 10^6$  chondrocytes per hour (30).

During the last 48 hours of the incubation period with hydrocortisone 10  $\mu\text{Ci}/\text{ml}$  of the radioactive label was included in the medium. Newly synthesized  $^{35}\text{S}$ -aggrecans partly accumulated in the artificial intercellular agarose matrix. Another part of these  $^{35}\text{S}$ -macromolecules escaped to the incubation medium. The agarose was mechanically disrupted and digested in the culture dish using 3 ml of 50 U/ml agarase (agarose 3-glycanohydrolase from *Pseudomonas atlantica*; Sigma) in 0.067 M phosphate pH 6.0 in the presence of proteinase inhibitors [0.1 M  $\alpha$ -amino-n-caproic acid, 0.01 M EDTA, 0.005 M benzamidinium-chloride and 0.01 M phenylmethylsulphonyl fluoride (31)] at  $40^{\circ}\text{C}$  overnight. The resulting suspension was centrifuged for 10 min. at 1000 rpm. The incubation medium and the supernatant were pooled at  $-20^{\circ}\text{C}$  for further chromatography.

After centrifugation, aliquots of pools of combined media and agarose digests were desalted through a Sephadex G25-column (Pharmacia; Uppsala, Sweden) chromatography gel in 0.067 M phosphate ( $\text{K}_2\text{HPO}_4/\text{Na}_2\text{HPO}_4$ ) pH 6.8, containing 0.01 M  $\text{Na}_2\text{SO}_4$  in order to separate the  $^{35}\text{S}$ -labelled aggrecans from free  $^{35}\text{S}$  sulphate. The eluted macromolecular fractions were counted for radioactivity. The radioactivity under the curves was related to the total incorporation of  $^{35}\text{S}$  sulphate in aggrecans by the respective cultures.

#### *Quantitative determination of total $^{35}\text{S}$ -labelled proteoglycans synthesized in alginate cultures*

After a 2-week culture period, as was the case for the agarose cultures, each test consisted of a 5-day (3 days plus 2 days) incubation period in the absence or presence of hydrocortisone. Hydrocortisone was diluted to the desired concentrations of 0.005  $\mu\text{g}/\text{ml}$  to 1  $\mu\text{g}/\text{ml}$ . Ten  $\mu\text{Ci}/\text{ml}$  of  $\text{Na}_2^{35}\text{SO}_4$  was included in the incubation medium of the alginate beads during the last 48 hours of hydrocortisone incubation. The culture medium was then aspirated and the alginate beads were washed and dissolved by incubation for

10 minutes with 3 ml of 55 mM sodium citrate pH 6.8, 0.15 M NaCl at 25°C. The resulting suspension was centrifuged at 900 rpm for 10 minutes to separate the cells with their cell-associated matrix [CAM; the pellet (23)] from the constituents of the interterritorial matrix (ITM; the supernatant). The CAM was further extracted by incubation for 48 hours under dissociative conditions with 4 M GuHCl in a 50 mM sodium acetate buffer pH 5.8 (32), at 4°C in the presence of proteinase inhibitors: 0.1 M  $\alpha$ -amino-n-caproic acid, 0.01 M EDTA, 0.005 M benzamidinium-chloride and 0.01 M phenylmethylsulphonyl fluoride (31). This solution was subsequently centrifuged for 10 min. at 1000 rpm and the dissociated CAM aggrecans were recovered in the supernatant. The suspensions obtained (CAM and ITM) and the nutrient media were desalted using Sephadex G25 column (Pharmacia) chromatography. In the 0.067 M phosphate buffer the dissociated aggrecans from the CAM were able to reassociate. The incorporation of  $^{35}\text{S}$  in the macromolecular fractions from the CAM, the ITM and the medium was calculated as mentioned above.

#### *Comparison between aggrecan synthesis by chondrocytes cultured in agarose and in alginate*

In order to investigate the correlation between aggrecan synthesis in the agarose and in the alginate culture system, chondrocytes from a 23-year-old male were cultured in 3-fold and in parallel in these 2 matrices (donor 9). The cultures were incubated for 5 days with two different concentrations of hydrocortisone (0.2  $\mu\text{g/ml}$  and 1  $\mu\text{g/ml}$ ) at day 14. A quantitative determination of the total  $^{35}\text{S}$ -labelled proteoglycans in the agarose system was performed as described above. A quantitative determination of the total  $^{35}\text{S}$ -labelled proteoglycans in the alginate culture system was obtained by adding the values for  $^{35}\text{S}$ -labelled proteoglycans in the CAM, in the ITM and in the nutrient medium.

#### *Chromatography of proteoglycans on Sepharose CL-2B analysis of the $^{35}\text{S}$ -aggrecan subpopulations*

Pools of the obtained nutrient media and

digests were desalted through a Sephadex G25 chromatography gel in order to separate  $^{35}\text{S}$ -labelled aggrecans from free  $^{35}\text{S}$ -sulphate. Fractions containing the  $^{35}\text{S}$ -labelled macromolecules were pooled and used for gel permeation chromatography on Sepharose CL-2B (Pharmacia) in the same buffer.  $^{35}\text{S}$ -labelled macromolecules have been shown to elute in three fractions.  $^{35}\text{S}$ -aggrecan aggregates and monomers separated from each other in the first 2 peaks (21, 33). Some low-molecular weight material, the exact nature of which has not been investigated (breakdown products; low-hydrodynamic size aggrecan subpopulations) eluted in the third tailing fraction. The radioactivity under the two first curves allowed to calculate the proportions of  $^{35}\text{S}$ -aggrecan aggregates and monomers.

#### *Statistics*

The experiments were conducted in 3-fold. Mean values and one standard deviation (1SD) were calculated. Statistical analysis was carried out by the unpaired two-tailed Mann-Whitney U-test to determine whether variables were significantly different ( $p < 0.05$ ) in the respective experiments. The Spearman rho-test was used for the correlation of the aggrecan synthesis in the two culture systems in one of the donors.

## **Results**

#### *Aggrecan synthesis in agarose culture*

The influence of hydrocortisone on  $^{35}\text{S}$ -aggrecan synthesis by human articular chondrocytes cultured in agarose is shown in Table I. Hydrocortisone doses ranging from 0.005  $\mu\text{g}$  to 1  $\mu\text{g/ml}$  induced a dose-dependent response result in the first 3 donors. The lowest concentration of hydrocortisone (0.005  $\mu\text{g/ml}$ ) augmented  $^{35}\text{S}$ -incorporation in donors 1 and 2:  $^{35}\text{S}$ -incorporation increased by  $\pm 40\%$  in 2 out of 3 donors. All of the higher doses resulted in a statistically significant and dose-dependent stimulation of aggrecan synthesis.

The hydrocortisone concentration was increased to 5  $\mu\text{g/ml}$  in donor 4 and to 1 mg/ml in donor 5. In donor 4, a plateau was reached at 1  $\mu\text{g/ml}$ : for doses of 1  $\mu\text{g/ml}$ , 2.5  $\mu\text{g/ml}$  and 5  $\mu\text{g/ml}$  an increase to nearly 200% of the control situation was observed. In donor 5, for concentrations of 500  $\mu\text{g/ml}$  and 1 mg/ml,  $^{35}\text{S}$ -incorporation dramatically decreased. However, only for the highest concentration of hydrocortisone (1 mg/ml) was the  $^{35}\text{S}$ -incorporation not significantly different from the control situation.

#### *Influence of hydrocortisone on aggrecan subpopulations in agarose cultures* Sepharose CL-2B gel permeation chro-

**Table I.** Effects of hydrocortisone on aggrecan synthesis by chondrocytes cultured in agarose (pg  $\text{SO}_4$  incorporated per hour per  $10^6$  cells).

	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	
Baseline aggrecan synthesis (absolute amounts)						
	9954 (6)	5576 (5)	1630 (7)	3195 (10)	3601 (20)	
% Change (relative amounts)						
Control	100% (6)	100% (5)	100% (7)	100% (10)	100% (20)	100% (10)
0.005 $\mu\text{g/ml}$	138% (1)	137% (3)	105% (11)*			127% (5)
0.05 $\mu\text{g/ml}$	187% (16)	122% (2)	123% (4)			144% (7)
0.2 $\mu\text{g/ml}$	177% (8)	181% (6)	162% (1)	174% (11)		174% (6)
1 $\mu\text{g/ml}$	246% (8)	212% (8)	183% (3)	207% (10)		212% (7)
2.5 $\mu\text{g/ml}$				188% (6)		
5 $\mu\text{g/ml}$				194% (6)	205% (12)	
20 $\mu\text{g/ml}$					198% (14)	
100 $\mu\text{g/ml}$					189% (5)	
500 $\mu\text{g/ml}$					122% (7)	
1 mg/ml					91% (20)*	

Donor 1: 2-year-old male; donor 2: 58-year-old male; donor 3: 65-year-old male; donor 4: 54-year-old female; donor 5: 61-year-old female. Data are presented as mean values of 3 cultures. Coefficient of variations are in brackets. Stimulation of  $^{35}\text{S}$ -incorporation is statistically significant ( $p < 0.05$ ) for all concentrations tested in all 5 donors, except for the lowest concentration in donor 3(\*) and the highest concentration in donor 5(\*).

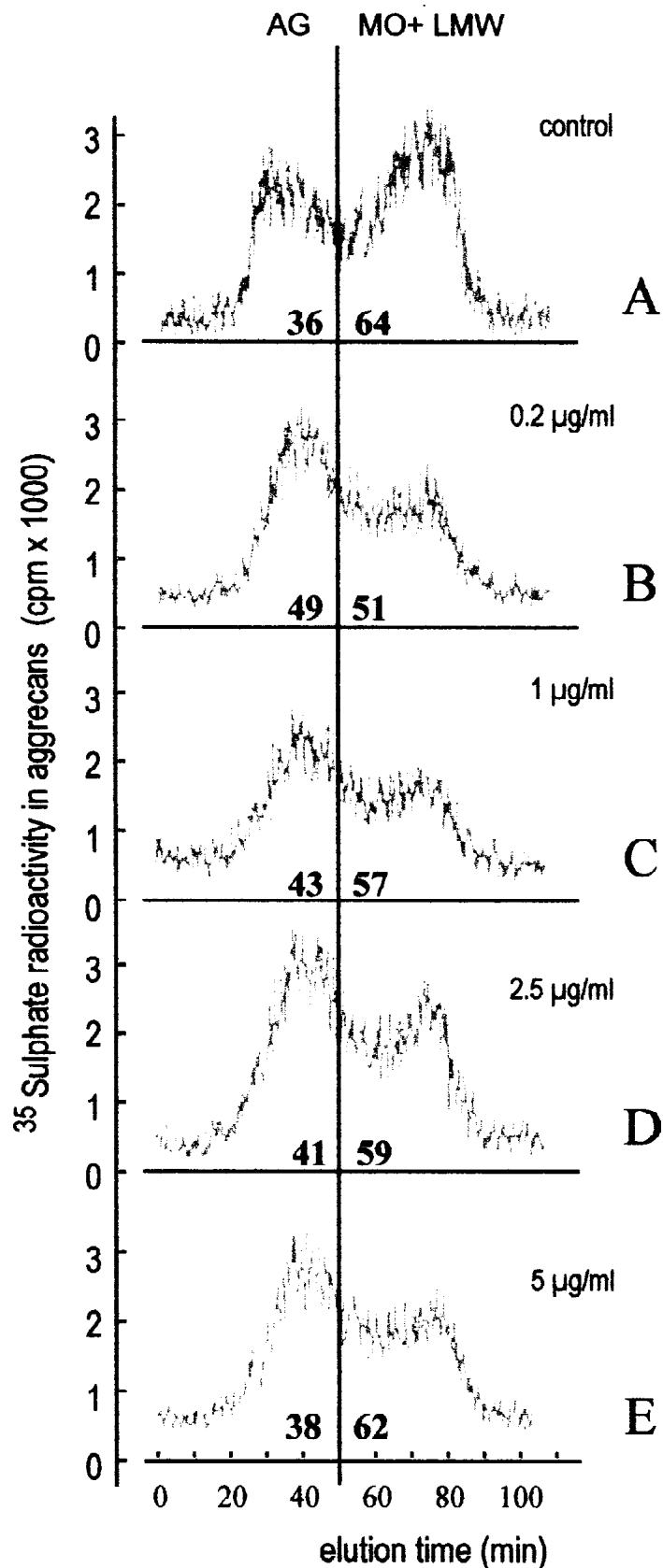
matography was performed on the pools of combined agarose digests and supernatant media obtained from the culture of each donor, in order to determine the influence of hydrocortisone on the quality of the newly synthesized aggrecans. The proportions of the aggrecan subtypes of the control situation in donor 4 are shown in Figure 1A. 36% of the  $^{35}\text{S}$ -labelled molecules are AG aggregates, while 64% are monomers and low molecular weight products. Under the influence of hydrocortisone at concentrations from 0.2  $\mu\text{g/ml}$ , the percentage of AG aggregates increased, while there were fewer monomers and low-molecular weight products. Higher concentrations of hydrocortisone (1  $\mu\text{g/ml}$  to 5  $\mu\text{g/ml}$ ) did not further increase the proportion of newly synthesized AG aggregates, (Fig. 1B -E).

#### Aggrecan synthesis in alginate culture

The influence of hydrocortisone on the accumulation of aggrecans in the different extracellular compartments investigated in the alginate culture system are listed in Table II. Compared to the control situation aggrecan synthesis was significantly increased when the chondrocytes were incubated with 0.005  $\mu\text{g/ml}$  up to 1  $\mu\text{g/ml}$  of hydrocortisone. This was true for the CAM and ITM. Donor 6, when incubated with 0.005  $\mu\text{g/ml}$  of hydrocortisone, showed a 21% increase in aggrecan synthesis in the CAM when compared to the control situation, while donors 7 and 8 showed increases of 82% and 25%, respectively. This dose-dependant increase was present at all the higher concentrations of hydrocortisone. Similar increases of aggrecan synthesis were also apparent in the ITM. In the nutrient medium this increase was not seen. In contrast,  $^{35}\text{S}$ -activity remained unaltered, possibly due to a lesser escape of aggrecan aggregates and monomers into this compartment when incubated with hydrocortisone.

#### Comparison of aggrecan synthesis in the two different matrices

Aggrecan synthesis in the agarose culture model incubated with 0.2  $\mu\text{g/ml}$  and 1  $\mu\text{g/ml}$  of hydrocortisone was 163% and 186%, respectively, compared to the control situation. In the alginate culture sys-



**Fig. 1.** Representative CL-2B profiles of  $^{35}\text{S}$ -labelled macromolecules, synthesized in the absence of hydrocortisone (control) and in the presence of 0.2  $\mu\text{g/ml}$ , 1  $\mu\text{g/ml}$ , 2.5  $\mu\text{g/ml}$  and 5  $\mu\text{g/ml}$  of hydrocortisone. The donor was a 54-year-old female. Numbers represent percentages of aggregates (AGGR), monomers (MO) plus low-molecular weight products (low-mol weight AG). AG = aggrecans.

**Table II.** Effects of hydrocortisone on aggrecan synthesis by chondrocytes cultured in alginate (pg SO<sub>4</sub> incorporated per hour per 10<sup>6</sup> cells).**A. Cell-associate matrix (CAM)**

	Donor 6	Donor 7	Donor 8	
	Baseline aggrecan synthesis (absolute amounts)			
	6682 (5)	2084 (10)	4462 (6)	
	% Change (relative amounts)			Mean changes (%)
Control	100% (5)	100% (10)	100% (6)	100% (6)
0.005 µg/ml	121% (1)	182% (7)	125% (4)	143% (4)
0.05 µg/ml	177% (4)	254% (7)	183% (3)	205% (5)
0.2 µg/ml	193% (4)	315% (3)	232% (2)	247% (3)
1 µg/ml	214% (2)	367% (4)	252% (3)	277% (3)

**B. Inter-territorial matrix (ITM)**

	Donor 6	Donor 7	Donor 8	
	Baseline aggrecan synthesis (absolute amounts)			
	7029 (7)	10648 (6)	12364 (3)	
	% Change (relative amounts)			Mean changes (%)
Control	100% (7)	100% (6)	100% (3)	100% (5)
0.005 µg/ml	115% (1)	130% (2)	118% (2)	121% (2)
0.05 µg/ml	119% (3)	127% (2)	122% (2)	123% (2)
0.2 µg/ml	139% (4)	142% (2)	132% (6)	138% (4)
1 µg/ml	226% (2)	147% (2)	135% (6)	169% (4)

**C. Nutrient medium**

	Donor 6	Donor 7	Donor 8	
	Baseline aggrecan synthesis (absolute amounts)			
	11319 (1)	5043 (4)	4180 (3)	
	% Change (relative amounts)			Mean changes (%)
Control	100% (1)	100% (4)	100% (3)	100% (3)
0.005 µg/ml	114% (2)	105% (3)	98% (2)	106% (2)
0.05 µg/ml	103% (10)	97% (5)	109% (8)	103% (8)
0.2 µg/ml	109% (2)	97% (6)	118% (2)	108% (3)
1 µg/ml	100% (2)	100% (5)	129% (3)	110% (3)

Donor 6: 25-year-old male; donor 7: 40-year-old male; donor 8: 24-year-old male. Data are presented as mean values of 3 cultures. Coefficient of variations are in brackets. Stimulation of <sup>35</sup>S-incorporation is statistically significant ( $p < 0.05$ ) for all concentrations tested in the 3 donors when compared to the control situation, and this for the CAM and for the ITM. In the medium no statistically significant difference ( $p < 0.05$ ) is found in the aggrecan synthesis rates when compared to the control situation.

tem (total aggrecan synthesis in the CAM, ITM and nutrient medium together) incubated with 0.2 µg/ml and 1 µg/ml of hydrocortisone, an increase of 39% and 44%, respectively, was seen when compared with the control situation. Using the Spearman rho-test a strong correlation in aggrecan synthesis between the agarose and the alginate culture system was found, with a correlation coefficient of 0.95 and a p-value of 0.007 (Fig. 2).

#### *Influence of hydrocortisone on aggrecan subpopulations in alginate cultures*

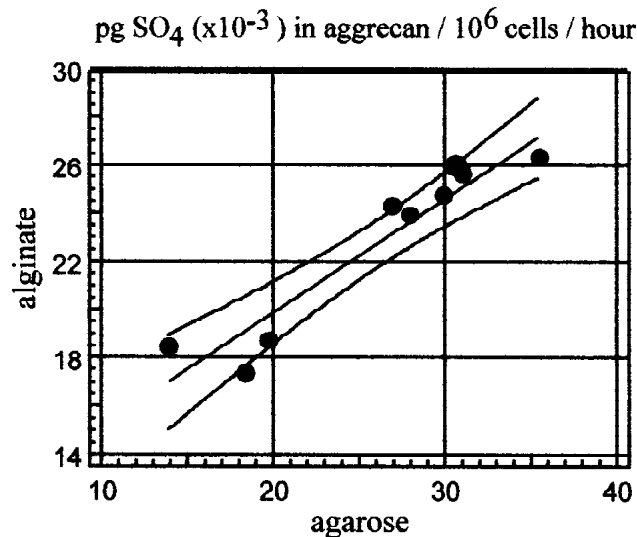
As in the agarose model, Sepharose Cl-2B gel permeation chromatography performed on the CAM of 3 alginate cultures from 3 different donors (donors 6, 7 and 8) after the dissociative condition showed significantly higher percentages of aggrecan in aggregates and in the monomeric form when pre-incubated with hydrocortisone. This was true at all con-

centrations of hydrocortisone used when compared with the control (Table III-A). In the ITM a significant increase of aggrecan in aggregates and in the monomeric form was also present, although the concentration of hydrocortisone that induced a significant increase was donor-dependent (Table III-B). In the nutrient medium, only the monomeric form of aggrecan showed an increase when the chondrocytes were pre-incubated with hydrocortisone but, as for the ITM, a donor-dependent difference in hydrocortisone concentration was required for a significant increase (Table III-C).

**Discussion**

These results demonstrate an important stimulatory effect of hydrocortisone on aggrecan synthesis by normal human articular chondrocytes. Data on the influence of glucocorticoids on human cartilage are scarce. AG synthesis was slightly reduced by hydrocortisone, 72.5 to 725 µg/100 ml, in one experiment on osteoarthritic cartilage organ explants (11). In another study, hydrocortisone was shown to inhibit sulphated glycosaminoglycan synthesis in human cartilage explants harvested from femoral heads during surgery (34). However, the drug effects in this experiment were found to vary significantly from one femoral head to another, probably due to the heterogeneity of the chondrocyte populations in the different samples and their different viability.

These problems are avoided when homogeneous cell populations of isolated chondrocytes in suspension cultures are used. The agarose and alginate culture model is a valid system for studying both the anabolism and catabolism of normal chondrocytes (21, 35). To investigate the influence of all concentrations of hydrocortisone in one experiment, too large a number of chondrocytes would have been required. The different test concentrations of hydrocortisone were therefore divided over several donors. Hydrocortisone doses in the present experiments were similar to physiological and therapeutic serum levels. 0.2 µg/ml is the normal morning serum peak level (8.00 a.m.). *In vitro* cultures of chondrocytes in agarose produced a dose-dependent response in aggrecan synthesis when in-



**Fig. 2.** Correlation of the  $^{35}\text{S}$ -aggrecan synthesis rates of chondrocytes from a 23-year-old male cultured in alginate and in agarose. **Abscissa:** aggrecan synthesis rates in the agarose system; **ordinate:** aggrecan synthesis rates in the alginate system. Aggrecan synthesis rates were expressed as pg of  $\text{SO}_4$  incorporated in aggrecan per  $1 \times 10^6$  chondrocytes per hour ( $n = 9$ ).

Linear regression analysis shows a strong correlation between agarose cultures and alginate cultures for  $^{35}\text{S}$ -aggrecan synthesis ( $r = 0.95$ ;  $p = 0.007$ ).

cubated with hydrocortisone at concentrations of  $0.005 \mu\text{g/ml}$  to  $1 \mu\text{g/ml}$ . This increase reached a plateau at higher doses (up to  $100 \mu\text{g/ml}$ ) with approximately 200% of aggrecan synthesis when compared with the control situation. Only with supratherapeutic levels of  $500 \mu\text{g}$  to  $1 \text{ mg/ml}$  did the plateau begin to decline, probably due to the toxic effects of hydrocortisone at these megadoses. When chondrocytes were incubated with hydrocortisone at concentrations of  $0.005 \mu\text{g/ml}$  to  $1 \mu\text{g/ml}$  this dose-dependent response in aggrecan synthesis was also seen in the CAM and in the ITM of the alginate culture system. However, in the nutrient medium of the latter culture matrix no change in the  $^{35}\text{S}$ -aggrecans was found with increasing hydrocortisone concentrations.

In the present study the low coefficients of variation (in general less than 10%)

**Table III.** Influence of hydrocortisone on aggrecan subpopulations of chondrocytes cultured in alginate. Dose-dependent response experiments.

**A. Cell-associate matrix (CAM)**

	% Aggregates			% Monomers			% Low MW		
	6	7	8	6	7	8	6	7	8
Control	32 (6)	28 (10)	29 (4)	30 (7)	30 (8)	33 (5)	38 (5)	42 (8)	38 (3)
$0.005 \mu\text{g/ml}$	37 (5)*	34 (4)*	35 (5)*	37 (3)*	36 (4)*	36 (5)	25 (8)*	34 (4)	28 (3)*
$0.05 \mu\text{g/ml}$	39 (5)*	35 (5)*	39 (5)*	38 (3)*	38 (5)*	38 (3)*	23 (13)*	27 (5)*	24 (7)*
$0.2 \mu\text{g/ml}$	39 (5)*	38 (4)*	39 (7)*	39 (3)*	39 (4)*	41 (5)*	22 (9)*	22 (4)*	20 (21)*
$1 \mu\text{g/ml}$	40 (4)*	38 (8)*	42 (6)*	41 (5)*	41 (8)*	40 (4)*	19 (16)*	21 (8)*	18 (13)*

**B. Inter-territorial matrix (ITM)**

	% Aggregates			% Monomers			% Low MW		
	6	7	8	6	7	8	6	7	8
Control	32 (3)	30 (10)	31 (8)	32 (5)	29 (9)	28 (10)	36 (2)	41 (4)	41 (2)
$0.005 \mu\text{g/ml}$	33 (5)	36 (3)*	35 (7)	33 (6)	36 (6)*	34 (8)	33 (10)	28 (8)*	21 (11)*
$0.05 \mu\text{g/ml}$	36 (3)*	38 (4)*	41 (4)*	34 (5)	39 (6)*	37 (6)*	29 (10)*	23 (9)*	22 (13)*
$0.2 \mu\text{g/ml}$	36 (2)*	40 (6)*	39 (6)*	36 (6)	41 (7)*	39 (6)*	29 (8)*	19 (4)*	22 (20)*
$1 \mu\text{g/ml}$	43 (4)*	40 (5)*	41 (6)*	41 (4)*	43 (7)*	40 (4)*	16 (20)*	17 (17)*	19 (15)*

**C. Nutrient medium**

	% Aggregates			% Monomers			% Low MW		
	6	7	8	6	7	8	6	7	8
Control	1 (60)	1 (36)	1 (30)	20 (13)	20 (20)	21 (12)	79 (3)	79 (5)	78 (3)
$0.005 \mu\text{g/ml}$	1 (27)	1 (33)	1 (29)	22 (16)	24 (9)	26 (5)	77 (4)	75 (3)	73 (2)
$0.05 \mu\text{g/ml}$	1 (27)	2 (27)	1 (20)	24 (9)	29 (14)	29 (8)	75 (3)	62 (7)	70 (3)*
$0.2 \mu\text{g/ml}$	1 (46)	1 (20)	1 (18)	25 (8)	35 (8)*	32 (11)	73 (3)	64 (4)*	64 (2)*
$1 \mu\text{g/ml}$	1 (38)	1 (22)	1 (20)	27 (6)*	36 (9)*	31 (8)	72 (2)*	63 (5)*	68 (4)*

Donor 6: 25-year-old male; donor 7: 40-year-old male; donor 8: 24-year-old male. Percentage of aggrecan subpopulations [aggrecan aggregates, monomeric form of aggrecans and low-molecular weight proteoglycans (low MW)] are presented as mean values of 3 cultures. Coefficient of variations are between brackets. The cell-associated matrix (CAM), the inter-territorial matrix (ITM) and the nutrient medium were investigated.

\*: a significant difference ( $p < 0.05$ ) when compared to the control situation.

in both matrices plead for the high reproducibility of chondrocyte suspension cultures. As already reported, a difference in the aggrecan synthesis rate depending on the age of the donor was present in the control situation (30). The aggrecan synthesis rate is decreased in an ageing population after maturity has been reached. These results can be extrapolated to *in vivo* synthesis rates. However, it may be assumed that articular chondrocytes keep their age-related *in vivo* synthesis properties in this culture condition.

Aggrecan synthesis in the two matrices, when stimulated with 0.2 µg/ml and 1 µg/ml, showed a strong correlation as demonstrated by the high correlation coefficient.

Simultaneously with an enhancement of AG-synthesis, hydrocortisone augmented the fraction of large AG (AG aggregates and AG monomers) released by the chondrocytes cultured in the two types of matrices. These findings are in agreement with earlier observations (18). Triamcinolone enhances the synthesis of large proteoglycans not only in normal but also in arthritic cartilage of C57Bl mice *in vivo* (36). Stimulation of glycosaminoglycan synthesis by hydrocortisone *in vitro* has been reported in rabbit chondrocytes (37).

Osteoarthritis occurs with an inflammation in the surrounding synovia. In this synovium an infiltration of lymphomyeloid cells is seen, of which some types produce substances which are extremely harmful to the joint tissues. Glucocorticoids inhibit the infiltration of these lymphomyeloid cells in the synovia, and thus reduce the effects of cytokines and suppress the metalloproteinase activity in cartilage and synovium.

The reluctance to use intra-articular steroid injections is mainly based on animal models indicating that these drugs may provoke or worsen cartilage lesions (1-6). However, studies on different models of experimentally induced OA and OA in humans reveal that corticosteroids may have beneficial effects on cartilage, as a result of different potential mechanisms. Firstly, corticosteroids suppress metalloproteinase activity in both cartilage (12, 17, 19) and synovium (38, 39). Secondly, glucocorticoids are potent

anti-inflammatory drugs in that they reduce interleukin-1 (40), thereby diminishing the harmful actions of these cytokines on chondrocytes. Finally, the results of the present study suggest a third mechanism, notably the stimulation of matrix (aggrecan) synthesis by chondrocytes. Therefore, glucocorticoids could play a part in the maintenance of homeostasis and the integrity of normal and diseased articular cartilage. Further research into this topic is required to clarify the influence of glucocorticoids on human articular chondrocytes, keeping in mind that not only the chondrocyte but the whole cartilage environment in normal and in arthritic joints could influence the effect of glucocorticoids on chondrocyte metabolism (41).

In conclusion, aggrecan synthesis, as measured by <sup>35</sup>S-incorporation by isolated normal human chondrocytes in suspension cultures, seems to be stimulated by the presence of hydrocortisone up to a concentration of 100 µg/ml. This applies to cultures in agarose and in alginate. When incubated with hydrocortisone the chondrocytes cultured in these two matrices show a strong correlation in aggrecan synthesis.

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