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Effects of Cyclic Hydrostatic Pressure on Proteoglycan Synthesis in Cultured Chondrocytes and Articular Cartilage Explants

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Primary chondrocyte cell cultures and explants of bovine articular cartilage were subjected to cyclic hydrostatic pressure in a novel computer-controlled pressure chamber designed for this purpose. The cultures were labeled with 5 μCi/ml $^{35}SO_4$ and simultaneously pressurized with 5 MPa load for 1.5 or 20 h with pressure cycles of 0.0167, 0.05, 0.25, and 0.5 Hz. The chondrocyte cell cultures were also subjected to 0.0082 and 0.0034 Hz cycles. Sulfate incorporation was significantly inhibited in cell cultures subjected to the 0.5, 0.25, or 0.05 Hz cyclic loads for 1.5 h, but stimulated in explant cultures with a 0.5 Hz cyclic 1.5-h load. Chondrocyte cultures subjected to longer (20 h) loading showed a stimulation of sulfate incorporation with 0.5 and 0.25 Hz cycles, but an inhibition with 0.0167 Hz. The results indicate that cyclic hydrostatic pressures of presumably physiological magnitude have significant influences on proteoglycan synthesis in articular cartilage chondrocytes. Comparison of the cell and explant cultures under identical pressure conditions suggested that chondrocyte interactions with extracellular matrix are involved in this regulation by cyclic hydrostatic pressure. The responses of the chondrocytes to pressurization also varied according to the total length of the treatment, a finding compatible with the idea of multiple metabolic steps in chondrocytes, both pre- and post-translational, controlled by the ambient hydrostatic pressure. © 1993 Academic Press, Inc.

The biomechanical properties of articular cartilage largely depend on its extracellular matrix, produced and maintained by the matrix-embedded chondrocytes. Under physiological conditions articular cartilage is subjected to various magnitudes and cycles of loading which control the matrix through the metabolic activity of chondrocytes. The biochemical and biophysical properties of articular cartilage vary at different surface sites according to joint loading (1). Moderate running exercise increases the thickness and glycosaminoglycan content of articular cartilage (2) whereas strenuous exercise can have an opposite influence (3). The *in vivo* models, as cited above, are relevant in testing the importance of loading for the properties of articular cartilage, but the mechanisms which lead to the changes are easier to study by aid of *in vitro* experiments.

The response of articular cartilage to loading is a complex phenomenon due to the numerous factors related to the mechanical strain of the tissue. At the beginning of the load application, a hydrostatic pressure gradient develops in the matrix, and then a portion of the interstitial fluid is driven out. The fluid escaping the matrix carries positive counterions and induces streaming potentials and currents (4). External compression of cartilage deforms chondrocytes (5). Decreased water content of cartilage leads to physicochemical changes in the matrix by increasing the osmotic pressure and fixed charge density and by reducing interstitial pH (6). Any of these phenomena may have biological consequences. Therefore, models in which the individual physical phenomena can be studied separately are important in revealing the cellular mechanisms of the joint loading response.

Numerous *in vitro* models have been developed to study the loading response of articular cartilage (7). In models using mechanical loading all of the above factors, i.e., hydrostatic pressure, deformation, streaming currents, and physicochemical changes, are working together. It is very difficult then to discern the mechanisms behind the alterations in important cartilage maintenance functions such as proteoglycan synthesis. We have earlier suggested

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that hydrostatic pressure is involved in the stimulation of articular cartilage PG[2] synthesis (8), but instruments for direct experimentation on cyclic hydrostatic pressure have not been available. The present paper describes the application of a novel pressure chamber capable of producing cyclic hydrostatic pressures of a magnitude and frequency close to those presumably existing in articular cartilage.

EXPERIMENTAL PROCEDURES

Materials

Materials for explant culture were obtained as described previously (9). Bovine knee joints were from the local abattoir (Itikka-Lihapolar, Kuopio, Finland). Fetal calf serum, basal medium Eagle (BME), and glutamine were from GIBCO Ltd. (Paisley, Scotland) and antibiotics from Flow Lab. (Irvine, Scotland). [^{35}S]-Sulfate (carrier free) was purchased from Amersham Int. (Little Chalfont, UK). All other reagents were obtained from Sigma (St. Louis, MO). The pressure chamber was constructed by Paineteho ky (Kuopio, Finland) and the hydraulic valves (pressure control and proportional regulator valves) were obtained by Robert Bosch GmBH (Stuttgart, Germany). The hydraulic pump was made by Vickers, Inc. (Troy, MI). The pressure transmitter with integrated pressure sensor, display, and relay output were from Hydac Electronics (Sulzbach, Germany). The Pt-100 resistance temperature sensor was from Signal-Anlagen, P. Bröckskes KG (Viersen, Germany). The peristaltic pump Posi-Flo I was obtained from Gelman Sciences (Ann Arbor, MI). The PC computer was manufactured by Osborne (Taiwan, Republic of China). Nitrocellulose membrane was from Millipore Corp. (Bedford, MA). High-molecular-weight hyaluronic acid, Healon, and Sephacryl S-1000 were purchased from Pharmacia (Upsala, Sweden). Surlyn and Bynel are registrated trademarks of Du Pont (Wilmington, DE), and Jet Melt from 3M United Kingdom PLC (Bracknell, UK), respectively. Measurements of pO_2, pCO_2, and pH were done with pH/blood analyzer (Instrumentation Laboratory System S.p.A., Milano, Italy).

Pressurizing System

The pressurizing system is composed of two chambers, a water-filling system, a hydraulic pump, and a hydraulic cylinder (Fig. 1). The center of the system is a thick-walled (wall thickness: 1.3 cm) cylindrical pressure chamber made of acid resistant steel (height: 41 cm; inner diameter: 8.5 cm). An equal-sized, nonpressurized reference chamber was a cylinder made of PVC plastic. Both chambers were filled by prewarmed (37°C) distilled water with a computer-controlled system. The hydrostatic pressure inside the pressure chamber was created by applying hydraulic technology. The oil flow generated by the hydraulic pump was conducted into the hydraulic cylinder, located under the pressure chamber, through a proportional regulator valve. The oil pressure was regulated by a proportional pressure valve. A rod attached to the piston of the hydraulic cylinder transferred the pressure into the pressure chamber, filled with distilled water. Both hydraulic valves (pressure control and proportional regulator valve) were driven by computer-controlled electronic amplifiers. The hydraulic system was equipped with water cooling of hydraulic oil to prevent heat conduction from the hydraulic cylinder to the pressure chamber.

Pressure measurement within the chamber was carried out using a solid state sensor integrated with a pressure transmitter and a display and pressure switch. A Pt-100 resistance sensor, installed in a protective tube, was used to measure the temperature of the pressure chamber. The apparatus, except the microcomputer, the hydraulic pump, and the control electronics, was assembled in a temperature-controlled room at 37°C.

The prewarmed distilled water could be automatically changed at selected intervals during experiments and circulated by a peristaltic pump through the pressure and reference chambers. Three computer-controlled valves regulated water change (Fig. 1). The peristaltic pump, valves, and pressure levels were controlled with a computer using programs generated by Turbo C language.

Loading procedure. In the pressure and reference chamber, 13 petri dishes (diameter: 35 mm) could be placed in separate racks. Before the experiment, the petri dishes were filled with the medium and sealed by covering with a Surlyn 1801-Bynel CXA 3048 bilayer membrane (thickness: 90 μm) after excluding all air. The membrane was attached to the petri dishes with Jet Melt 3764 adhesive. The cultures were labeled by injecting radiolabel by syringe through the membrane. The membrane was patched up by a small piece of tape and parallel dishes were immediately incubated at 37°C in the pressure and reference chambers.

Culture Conditions

Explant cultures. Articular cartilage tissue cultures were established from 1- to 2-year-old bovine knee joint cartilage. The knee was opened under sterile conditions and five consecutive cartilage plugs (ϕ 8 mm) were taken by punch from both sides of the patellar surface of femur (10). By aid of a scalpel, the uncalcified cartilage was separated from the subchondral bone and the wet weight of the explant was determined. After bonding with a tissue adhesive on the petri dishes, the explants (mean thickness: 1.5 mm) were cultured for 2 days at 37°C in Eagle's minimum essential medium with Earle's salts, supplemented with antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin), 70 μg/ml ascorbate, and 3 mM glutamine. After this 2-day preincubation, the explant cultures were covered with the membrane, labeled with 5 μCi/ml of $^{35}SO_4$, and pressurized at 5 MPa for 1.5 h using 1 s load/1 s unload (0.5 Hz), 1 s load/3 s unload (0.25 Hz), 1 s load/19 s unload (0.05 Hz), or 1 s load/59 s unload (0.0167 Hz) cycles. After incubation, the cartilage explants were digested with 0.1% papain. Sulfate incorporation was determined by precipitation of PGs with Safranin O onto a nitrocellulose membrane in a filtration manifold and exposing an autoradiographic film against the dried membrane (11). The mean density of the precipitation dots was measured by an image analysis system and calibrated with standard PGs of known radioactivity (11). For each plug, sulfate incorporation was calculated as pmol/mg wet weight/h. Each pressurized plug was compared to the average value of two plugs originating from the adjacent joint surface sites of the pressurized plug (12). Finally, the results were expressed as values related to the control level (pressurized/control-ratio).

Cell cultures. Bovine articular cartilage from the patellar surface of femur was cut into 1- to 2-mm^2 pieces followed by a sequential digestion by trypsin and crude collagenase. Cells were plated at 400,000 cells/ml on petri dishes (ϕ 35 mm) and cultured in BME with 25 mM Hepes and Hanks' salts without glutamine and supplemented with 10% fetal calf serum, 3 mM glutamine, antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin, 2.5 μg/ml amphotericin) at 37°C. The medium was changed every second day. After reaching confluence in 1–2 weeks, the cell cultures were covered with the membrane, labeled with 5 μCi/ml of $^{35}SO_4$ and pressurized at 5 MPa for 1.5 h or 20 h using 1 s load/1 s unload (0.5 Hz), 1 s load/3 s unload (0.25 Hz), 1 s load/19 s unload (0.05 Hz), or 1 s load/59 s unload (0.0167 Hz) cycles. The chondrocytes were also subjected to 1 s load/2 min unload (0.0082 Hz) and 1 s load/5 min (0.0034 Hz) unload cycles for 20 h. After pressurization, the dishes were put on ice and the medium was removed. The cells were dissolved in a solution containing 0.5% Chaps, 50 mM Tris–HCl, 10 μM EDTA, and 4 M guanidium hydrochloride, pH 8.0. The DNA content in every dish

[2] Abbreviations used: PG, proteoglycan; BME basal medium Eagle; SDS, sodium dodecyl sulfate; Chaps, 3-[(3-cholamidopropyl)-dimethylammonio]propanesulfonic acid.

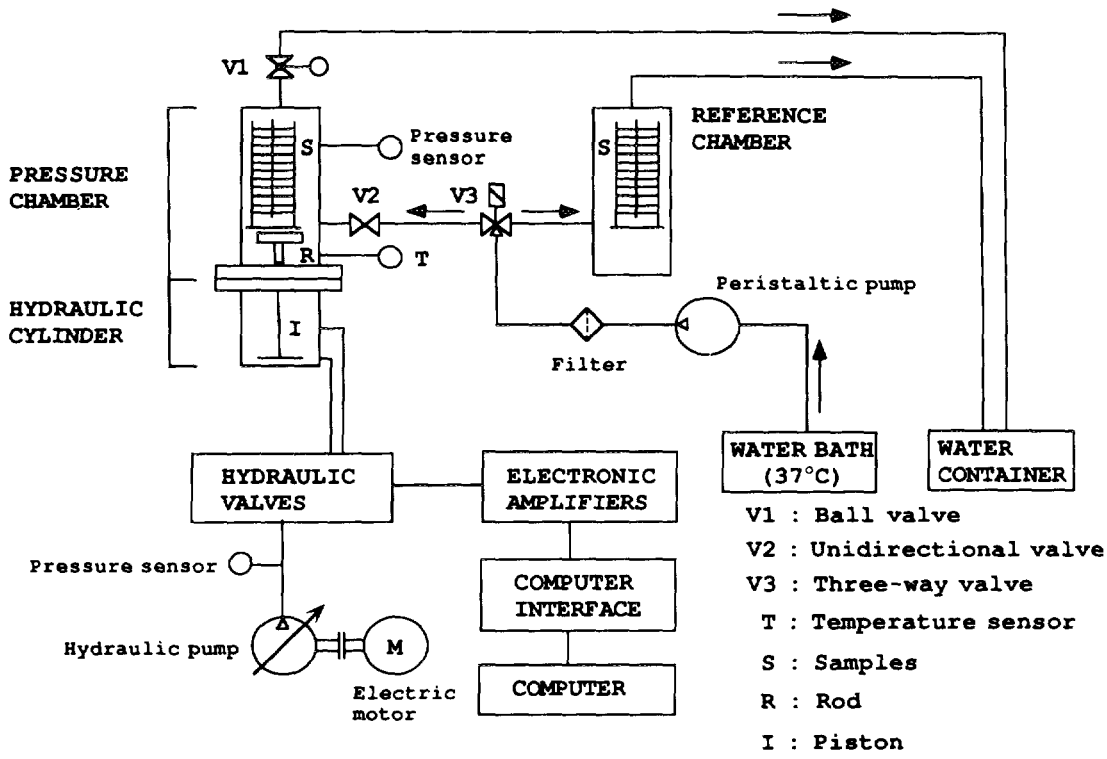


FIG. 1. A diagram of the pressure system. The sealed petri dishes are placed within specially designed rack (S) into the pressure chamber, located in the upper part of a stainless steel cylinder. A hydraulic cylinder is under the pressure chamber. The oil flow generated by the hydraulic pump is conducted into the hydraulic cylinder through hydraulic valves (proportional pressure control and proportional regulator valve). The pressure of the oil is regulated by a proportional pressure valve. Both valves are driven by computer-controlled electronic amplifiers. A rod (R) attached to the piston (I) of the hydraulic cylinder transfers the pressure into the pressure chamber, filled with distilled water. A peristaltic pump is used to fill the chambers and to circulate the water through the pressure and reference chambers. The selection between the chambers is made by the three-way valve V3. The water change of the pressure chamber is made via the unidirectional valve V2 and air-actuated ball valve V1. The valves V1 and V2 isolate the pressure chamber from the other parts of the water change system. The arrows show the direction of the prewarmed distilled water.

was measured from the cell fraction (13). Sulfate incorporation was determined with the same method as with the explants above. To normalize the difference between the dishes, the results were calculated as pmol/μg DNA/h for every dish. The final results were expressed as pressurized/control-ratio.

Evaluation of culture conditions during loading. A temperature transducer was inserted into a petri dish to monitor the temperature changes in the medium during sealing of a dish with a Surlyn membrane. In addition, concentration of O_2, CO_2, and pH was measured in the membrane-sealed and lid-covered (control) dishes, containing confluent chondrocyte cultures, on Days 0, 1, 2, and 3 after covering of the dishes with the membrane.

SDS-agarose gel electrophoresis. Media from cell cultures containing 20,000 dpm of incorporated $^{35}SO_4$ were precipitated overnight at −20°C with 3 vol of 0.5% (w/v) sodium acetate in 94% (v/v) ethanol and washed three times with 0.5% (w/v) sodium acetate in 80% (v/v) ethanol. Samples were then electrophoresed on SDS-agarose gel as described earlier (14).

Sephacryl S-1000 gel filtration chromatography. The ability of secreted PGs to aggregate was studied after incubation with high-molecular-weight hyaluronan. Nasal cartilage PGs (100 μg) and medium samples (50,000 dpm of incorporated $^{35}SO_4$/ml) were combined and diluted with gel filtration elution buffer (0.1% Chaps, 0.15 M NaCl, 50 mM $NaSO_4$, 5 mM Na_2-EDTA, 10 mM benzamidine, 0.03% (w/v) NaN_3). Aggregation was accomplished overnight at +4°C with the addition of 4 μg exogenous hyaluronan. The samples were then frozen and chromatographed immediately after thawing on Sephacryl S-1000. Radioactivity of the fractions was determined by the Safranin O precipitation assay (11).

Statistical Analysis

Student's unpaired (cell) or paired (explants) two-tailed *t* test was used for statistical analysis. *P* values less than 0.05 were considered significant.

RESULTS

The main parts of the novel apparatus constructed for the exposure of cultured cartilage cells and explants to hydrostatic pressure are described in Fig. 1. It produces both static and cyclic loads, with a wide range of cycle lengths. The lengths of the loading and the unloading periods in the cyclic mode are freely selectable. The pressure in the chamber can be chosen with 0.1 MPa intervals between 4 and 32 MPa (Fig. 2). The velocity of the rise and fall of the pressure can be set in the computer program

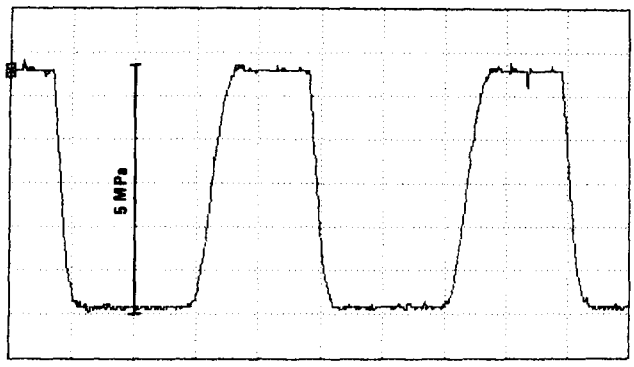


FIG. 2. The cyclic pressure in the pressure chamber as seen on the oscilloscope screen. The duration of the cycle was set to 0.5 Hz and the pressure to 5 MPa. Abscissa, 500 ms/div.

by the control voltage of the proportional regulator valve, because the oil flow velocity in proportional regulator valve is directly related to the rise and fall of the pressure. By the settings used in the present experiments, the rise time up to 5 MPa (from 10 to 90% of the level of selected pressure) was 200 ms, i.e., the rise velocity was 18 MPa/s. The fall time was 130 ms and the velocity 31 MPa/s. The maximum rise and fall times were quicker if the pressure level was increased. With 30 MPa pressure, the velocities were 57 MPa/s (rise time) and 92 MPa/s (fall time). During loading, the pressure was monitored by a sensor in the chamber and kept at the predetermined level with the accuracy of 0.1 MPa.

Subjecting chondrocyte cultures to hydrostatic pressure was done by immersing the membrane-covered culture dishes in a water-filled pressure chamber. Water is virtually incompressible allowing a rapid, reproducible transduction of the pressure from the water to culture medium and the cells. Any gas phase remaining inside the membrane is readily compressed and more or less dissolved in the medium at elevated pressures. The apparatus withdraws automatically its actions if the pressure level elevation is reduced due to the presence of air in the pressure chamber. Therefore, all air was carefully excluded from the tissue culture dishes before sealing.

A leak-free junction between the membrane and the rim of the dish was obtained with the Jet Melt adhesive. The bonding occurs at an elevated temperature, but monitoring the liquid within the dish showed that the rise was maximally 6°C from the initial (room) temperature. There was no signs of heat injury or toxic influences on the cells due to the membrane attachment procedure.

Different materials were tested to cover the petri dishes. The most suitable ones were certain types of plastics (Surlyn). Without the gas phase the cultures have only a limited quantity of oxygen available for their metabolism which may disturb long term experiments. Therefore, the partial pressures of O_2, CO_2, and pH were measured in sealed and lid-covered (control) dishes. In control dishes with 5% CO_2 in the atmosphere, the pO_2 was only slightly decreased (Fig. 3). When the dishes were sealed with the Surlyn membrane, oxygen pressure decreased on the average from 21 to 11 mmHg during 3 days (Fig. 3). The partial pressure of CO_2 increased in both dishes, but more in the membrane-covered dish. However, pH values were similar in covered and uncovered dishes (Fig. 3).

We investigated the influences of hydrostatic pressure on both cartilage explant and chondrocytes cultures. The explants were exposed to 5 MPa hydrostatic pressure for 1.5 h with four different cycles and simultaneously labeled

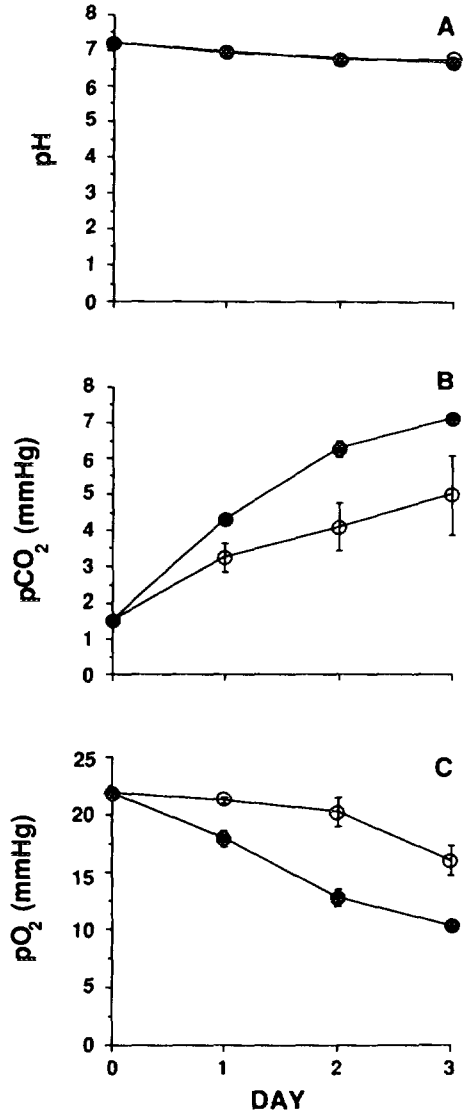


FIG. 3. pH (A), pCO_2 (B), and pO_2 (C) in confluent chondrocyte cultures in petri dishes kept in 5% CO_2 atmosphere (○, uncovered) or covered with Surlyn membrane (●) for 0–3 days at 37°C. Mean ± SD (n = 6–7).

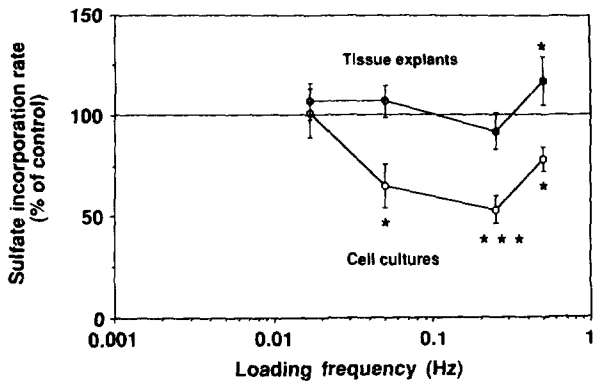


FIG. 4. Sulfate incorporation in explant and cell cultures of bovine articular cartilage subjected to 5 MPa pressure with cycles 0.5, 0.25, 0.05, and 0.0167 Hz for 1.5 h, expressed as % of unpressurized control cultures in the reference chamber. The values were calculated as pmol/μg DNA/dish (explants) or pmol/μg DNA/dish (cell cultures). Explants, mean ± SE of 14–16 explant pairs in each point from four animals, four separate experiments in each data point (one experiment/one knee). Cell cultures, mean ± SE of 9–12 cultures in each point. $^{*}P < 0.05$; $^{***}P < 0.001$.

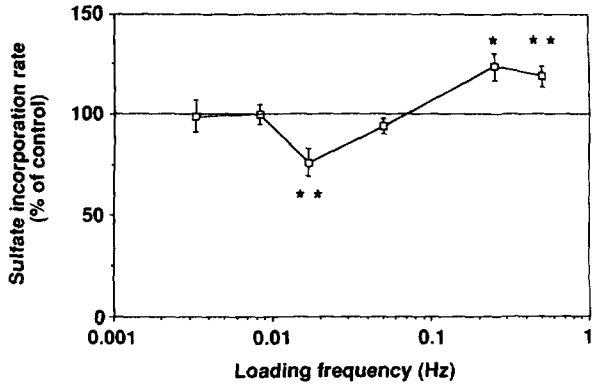


FIG. 5. Sulfate incorporation in cell cultures of bovine articular cartilage subjected to 5 MPa pressure with 0.5, 0.25, 0.05, 0.0167, 0.0082, and 0.0034 Hz cycles for 20 h. The figures were expressed as % of unpressurized control cultures in the reference chamber after determination of the pmol/μg DNA/dish (mean ± SE of 24–36 cultures and two separate experiments in each point except three separate experiments with 0.0167 Hz. $^{*}P < 0.05$; $^{**}P < 0.01$).

with $^{35}SO_4$ to determine the proteoglycan synthesis rate. The mean [^{35}S]sulfate incorporation in the six control sites was 9.5 ± 1.0 (SE) pmol/mg wet weight/h. There was no statistically significant differences in sulfate incorporation between the control sites. Sulfate incorporation was stimulated (+17%, 1.5 pmol/mg wet weight/h) by 0.5 Hz cyclic pressure ($P < 0.05$). With the other cycles used (0.25, 0.05, or 0.0167 Hz), there were no statistically significant changes in the incorporation rate (Fig. 4). The respective values with the other cycles were 0.6 pmol/mg wet weight/h inhibition (0.25 Hz), 0.8 pmol/mg wet weight/h stimulation (0.05 Hz), and 0.7 pmol/mg wet weight/h stimulation (0.0167 Hz).

Chondrocyte cell cultures subjected to identical cycles for 1.5 h showed a significantly inhibited sulfate incorporation with 0.5 Hz ($P < 0.05$), 0.25 Hz ($P < 0.001$), and 0.05 Hz ($P < 0.05$) cycles (Fig. 4). However, when the loading time was increased from 1.5 to 20 h, sulfate incorporation was stimulated with 0.5 Hz ($P < 0.01$) and 0.25 Hz cycles ($P < 0.05$), but inhibited with the 0.0167 Hz cycle ($P < 0.01$). When the interval between pressure impulses was further increased to 0.0034 Hz, there was no statistically significant alteration in sulfate incorporation (Fig. 5). With the 0 s/0 s cycle (no pressure in either chamber) no significant differences existed between the pressure and reference chambers.

The chondrocytes were maintained in primary monolayer culture before pressurization. SDS–agarose gel electrophoresis of newly synthesized proteoglycans showed that the cells produced mainly aggrecans and less of the small proteoglycans (Fig. 6). A large proportion of the PGs were able to aggregate with hyaluronan (Fig. 7). There were no major changes in PG size and ability to aggregate due to loading (Figs. 6 and 7). The changes in sulfate incorporation were not due to an altered number of cells, because all results were calculated per DNA content. Furthermore, there were no statistically significant differences in DNA content between the control and the loaded dishes.

DISCUSSION

In this study we describe an apparatus constructed for hydrostatic loading of articular cartilage explants and chondrocytes and demonstrate that cyclic hydrostatic pressure influences the proteoglycan synthesis rate in both culture systems. Hydrostatic pressure is one of the several physical and physicochemical factors which operate in

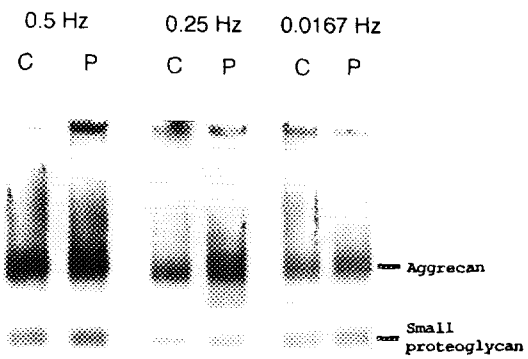


FIG. 6. Fluorogram of an SDS–agarose gel electrophoresis of $^{35}SO_4$-labeled PGs secreted by the chondrocyte cultures. The migration positions of aggrecan and small PGs are indicated in the control cultures (C) and those pressurized (P) at 5 MPa with 0.5, 0.25, and 0.0167 Hz cycles.

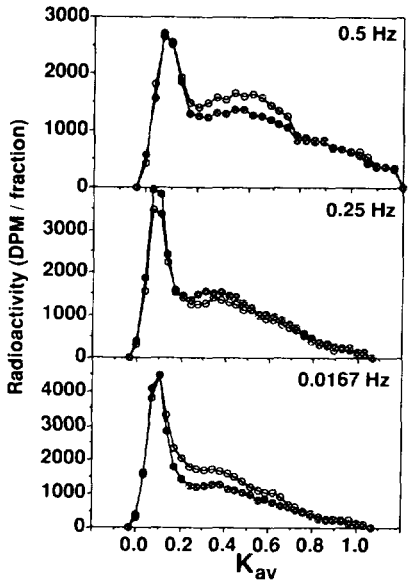


FIG. 7. Associative Sephacryl S-1000 gel chromatography of PGs synthesized by cultured articular cartilage chondrocytes. Nasal cartilage PGs (100 μg) and hyaluronan (4 μg of Healon) were added as a carrier to labeled PGs from the culture medium and incubated overnight at +4°C before chromatography. Radioactivity in control (open circles) and experimental samples (closed circles) was determined by Safranin O precipitation as described under Experimental Procedure.

articular cartilage subjected to loading. Among these factors, only hydrostatic pressure can be studied in a relatively "pure" form, independent of the others. Hydrostatic pressure induces no fluid flow or tissue deformation, which would bring about the other factors. We have observed stimulation of proteoglycan synthesis in cartilage explants mechanically compressed with a nonporous stainless steel loading head. It was found that a 0.5 MPa load of 50 ms duration repeated at 4-s intervals induced the highest $^{35}SO_4$ incorporation (8, 15). With that kind of cyclic compression, very little deformation or fluid flow occurred in the cartilage plugs. This suggested that pulses of hydrostatic pressure could be responsible for the stimulation.

Oxygen reaches the tissue only by diffusion. This may cause the O_2 saturation of the cartilage to drop below 1% (7.6 mmHg) (16). The importance of oxygen concentration in cartilage metabolism is not completely understood as yet, but articular cartilage appears to have a broad tolerance for different oxygen tensions as indicated by unchanged $^{35}SO_4$ incorporation in cultures with 5 to 60% O_2 in the surrounding atmosphere (17). It is unlikely that the oxygen decrease in the membrane-covered cultures severely interferes at least during the first day, since a comparable O_2 reduction was observed after 2–3 days even in control cultures. The pCO_2 was increased during the cell culture, as expected, but the difference between the covered and control dishes remained small enough to cause no change in the pH of the Hepes-buffered medium. The Surlyn membrane is partly permeable to O_2 and CO_2, but not to water or its other solutes. In the present system, the membrane thus seems to be nontoxic, be easily applied, and retard but not completely inhibit the exchange of gases.

Numerous *in vitro* models have been developed for cartilage loading, including mechanical compression (6, 9, 18–23), stretching (24, 25), and hydrostatic pressure (12, 26, 27). Earlier *in vitro* studies have shown that the response of cartilage for loading depends on whether dynamic (cyclic) or static loading is applied on cartilage. Cyclic compressive loading led to an inconsistent response of cartilage, depending on loading protocol (20–22), whereas static compressive loading consistently inhibits proteoglycan synthesis in cartilage (6, 18, 20–23).

Thus far, hydrostatic loading experiments have yielded partly inconsistent results. A low level continuous hydrostatic pressure (5.8 kPa) inhibited the accumulation of cAMP and enhanced the cellular uptake of radiolabeled calcium in chick tibial epiphyseal cartilage (28) while in another study 13 kPa pressure with a 0.3 Hz cycle increased cAMP levels in explants and cells of epiphyseal cartilage (29). In the same model with chick epiphyseal cells and explants, intermittent pressurization increased proteoglycan synthesis by 40 and 120%, respectively. In addition, the amount of nonextractable proteoglycans increased (26). Relatively higher, but presumably physiological levels of static hydrostatic pressure up to 2.75 MPa had no effect on proteoglycan synthesis by cultured bovine articular cartilage (30). On the other hand, static hydrostatic pressures between 75 and 300 psi (0.5–2.1 MPa) inhibited sulfate incorporation by 21–53%, but 375 psi (2.6 MPa) stimulated $^{35}SO_4$ incorporation by 10–15% (27). Hydrostatic pressure (13 kPa) increased calcium phosphate deposition in the cartilage matrix, intermittent pressure being more effective than static loading (29). It also increased $^{35}SO_4$ incorporation into fetal bone rudiments (31, 32). A dual effect was found in bovine articular cartilage slices exposed to 5–50 MPa hydrostatic pressure: a stimulation of proteoglycan synthesis after a single, short application of moderate pressure, and an inhibition with high (about 20–50 MPa) continuous pressures (12). Effects of cyclic hydrostatic pressures within high physiological levels on cultured cartilage explants or chondrocytes have not been studied earlier.

The pressures used in the present experiments are within the physiological range found in human joints. Peak pressures up to 18 MPa have been measured in the human hip joint (33) but 5 MPa was the estimated pressure level in the knee joint during walking (34). The present study was performed with articular cartilage and chondrocytes from bovine knee joint. However, the pressures observed in joints of species of different size were relatively constant (35).

The response to short-term hydrostatic pressurization varied depending on whether primary chondrocytes or cartilage explants were used. Sulfate incorporation was significantly stimulated in explants when a rapid (0.5 Hz) cycle was applied, but inhibited with same protocol applied to chondrocytes in cell culture. An identical, dual response to hydrostatic pressure was earlier noticed when cartilage explants and chondrocyte suspensions were exposed to 5–15 MPa for 5 min (36). The cell–matrix interaction or the shape of the chondrocytes may be important in determining the response to short-term cyclic hydrostatic pressure. Cultured chondrocytes are more flattened in shape and surrounded by a less extensive and less organized matrix than their counterparts in explants.

The long-term experiments (20 h) were performed on chondrocyte cultures due to the strong (inhibitory) reactions found in the short-term (1.5 h) experiments. However, the response of the cells in the 20-h pressure experiments was totally reversed; a stimulation in sulfate incorporation was found with the same cycles and pressures that were inhibitory in short-term experiments. It seems that cyclic hydrostatic pressure influences several metabolic steps in chondrocyte metabolism, in either a stimulatory or an inhibitory way, and the balance determines the net synthesis of proteoglycans. The time for complete aggrecan molecule synthesis takes about 1.5–2 h, consisting of core protein translation and transfer from the rough endoplasmic reticulum to the Golgi apparatus in about 70–90 min, followed by glycosaminoglycan chain elongation, sulfation, and secretion in about 15 min (37). In our short-term experiments with 1.5 h pressure it thus seems that most, if not all alterations observed in $^{35}SO_4$ incorporation must have occurred during translation or post-translational processing, whereas in the long-term (20 h) experiments hydrostatic pressure may have altered also gene expression.

In conclusion, the present work indicated that cyclic hydrostatic pressure alone has a controlling effect on proteoglycan synthesis in both cartilage explants and cultured chondrocytes. Both stimulatory and inhibitory responses were induced, depending on the frequency of the pressure, the length of pressurization, and the state of the chondrocytes, whether in intact tissue or in the monolayer. It is suggested that hydrostatic pressures of physiological magnitude have multiple impact points on chondrocyte metabolism. The present experimental system will be used to characterize them in more detail.

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