Biochemical Pharmacology, Vol. 37, No. 19, pp. 3683–3690, 1988.
Printed in Great Britain.

0006–2952/88 $3.00 + 0.00

INHIBITION OF GLYCOSAMINOGLYCAN SYNTHESIS IN ANATOMICALLY INTACT RAT PATELLAR CARTILAGE BY PARACETAMOL-INDUCED SERUM SULFATE DEPLETION

PETER M. VAN DER KRAAN,* BERNARD J. DE VRIES, ELLY L. VITTERS, WIM B. VAN DEN BERG and LEVINUS B. A. VAN DE PUTTE

Department of Rheumatology, Academic Hospital St. Radboud, University of Nijmegen, Geert Groote plein Zuid 8, 6525 GA Nijmegen, The Netherlands

(*Received* 24 *November* 1987; *accepted* 14 *April* 1988)

Abstract—We have studied the effect of low sulfate concentrations on the glycosaminoglycan synthesis in rat patellar cartilage *in vivo* as well as *in vitro*. The oral administration of 200 mg/kg paracetamol to male Wistar rats resulted in a significant reduction of the serum sulfate concentration. Reduced serum sulfate availability resulted in a 34% decrease of glycosaminoglycan synthesis in patellar cartilage. This is due to sulfate depletion since paracetamol had no direct effects on glycosaminoglycan synthesis and a slight but significant inhibitory effect on the catabolism of radiolabeled glycosaminoglycans *in vitro*.

The glycosaminoglycans synthesized at low sulfate concentrations *in vivo* were similar to the glycosaminoglycans synthesized at physiological sulfate concentrations. Studying the effect of sulfate availability *in vitro* on glycosaminoglycan synthesis in patellar cartilage we found that incubation of rat patellae in medium containing less than 0.5 mM inorganic sulfate led to a decreased sulfate incorporation. The use of potential sulfate decreasing drugs can lead to inhibition of glycosaminoglycan synthesis. This argues for a reconsideration of the use of these drugs in patients with already dysfunctioning cartilage metabolism as in rheumatoid arthritis and osteoarthrosis.

Nonsteroidal antiinflammatory drugs (NSAIDs) are used in the treatment of both rheumatoid arthritis and osteoarthrosis (osteoarthritis). These diseases are associated with the pathology of articular cartilage. However, in the case of osteoarthrosis cartilage destruction is a primary event while in rheumatoid arthritis it is a secondary phenomenon following the inflammation of synovial tissue. Several investigations have demonstrated that certain NSAIDs might affect the biochemical properties of articular cartilage chondrocytes.

NSAIDs can exert their pharmacological effects on chondrocytes in two ways: a direct effect on the biochemical activities of chondrocytes and/or an indirect effect by way of influencing systemic factors determining the function of chondrocytes. Many direct effects of NSAIDs are reported in the literature [1–7]. Salicylates are reported, by Palmoski and Brandt, to suppress glycosaminoglycan synthesis both *in vitro* and *in vivo* [8–11]. These authors showed that this effect was a direct effect of salicylate on the chondrocyte metabolism in their experiments [12]. An indirect effect of salicylate on glycosaminoglycan synthesis in articular cartilage has been observed in our experiments [13, 14]. A decrease of serum sulfate concentration in mice by way of salicylate-induced sulfate diuresis, apparently was the causative factor producing a diminished synthesis of glycosaminoglycans in anatomically intact patellar cartilage [13, 14].

Herein we describe the effect of serum sulfate reduction on cartilage glycosaminoglycan synthesis in more detail. We used paracetamol instead of salicylate, to decrease sulfate availability. In this way possible direct effects of salicylate on glycosaminoglycan synthesis are eliminated. Paracetamol is conjugated with sulfate by rat liver enzymes resulting in depletion of serum sulfate [15]. We used in this study Wistar rats instead of mice because rats are relatively insensitive to paracetamol-induced hepatoxicity and have a higher capacity of paracetamol sulfoconjugation than mice [16, 17].

MATERIALS AND METHODS

Effect of paracetamol on the serum sulfate concentration. Male Wistar rats (150–200 g) were used in all experiments. They were fed with a commercial pellet diet and given fresh tap water *ad libitum*. Paracetamol (200 mg/kg) dissolved in tap water (1 ml) was orally administered to the rats. Control animals received a comparable volume of only tap water. After 2, 5, 7 and 24 hr a blood sample was taken by orbita-punction under a light ether anesthesia. Inorganic sulfate was determined by a modification of the benzidine method of Dogson and Spencer [18] as recently described [19].

Effect of paracetamol and salicylate on glycosaminoglycan synthesis in vitro. Wistar rats were killed by cervical dislocation and both intact patellae were, with a standard amount of surrounding tissue, dissected from the knee joints. Patellae were incubated in RPMI 1640 DM medium supplemented with 2 mM L-glutamine and 1 mM

* To whom correspondence should be addressed.

pyruvate (all from Flow Laboratories, Irvine, Scotland). The sulfate concentration in the medium was 0.6 mM. Various concentrations of paracetamol and sodium salicylate (1, 2 and 5 mM) were added to the medium at the initiation of the 4-hr incubation period. Administration of 200 mg/kg paracetamol to male Wistar rats (150–200 g), as in our experiments, will result in a maximum serum paracetamol concentration of 0.2–0.3 mM [15]. After 2 hr incubation, 20 μCi [^{35}S]sulfate (Radiochemical Centre, Amersham, U.K.) was added to the medium. All incubations were performed at 37° in a humidified atmosphere containing 5% CO_2. Patellae were washed twice with physiological saline and subsequently fixed in ethanol (96%, v/v). Decalcification of the patellae with 5% (v/v) formic acid was followed by stripping of the patellar cartilage layer [19]. Patellar cartilage was digested overnight at 60° by solulyte (J. T. Baker Chemicals, Deventer, The Netherlands) and the amount of incorporated [^{35}S]sulfate per cartilage of one patella was assayed by liquid scintillation analysis.

Effect of paracetamol on the catabolism of [^{35}S]sulfate labeled glycosaminoglycans. Patellae were incubated in culture medium containing 80 μCi [^{35}S]sulfate/ml for 2 hr as described above. This labeling period was followed by a chase without radiolabel for 16 hr in medium containing 0, 1, 2 or 5 mM paracetamol. This was succeeded by processing of the patellae as described above.

Effect of medium sulfate concentration on glycosaminoglycan synthesis in vitro. Rat patellae were incubated in BME-diploid medium (Flow Laboratories) with various sulfate concentrations for 2 hr at 37° in humidified 5% CO_2 atmosphere. The BME medium was supplemented with 2 mM L-glutamine, 1 mM pyruvate, 20 μCi [^{35}S]sulfate (carrier-free) and 40 μCi D-[6-^{3}H]glucosamine (RCA) per ml, respectively. Patellar cartilage was isolated from the surrounding tissue as described above and glycosaminoglycans were isolated. To this end patellar cartilage was digested overnight by papain at 60°. The papain mixture (pH 6.0) consisted of 1 mg/ml papain (type IV, double crystallized, Sigma, St. Louis, MO, U.S.A.), 0.1 M Na-acetate, 10 mM L-cysteine hydrochloride and 50 mM Na_2-EDTA. Non-hydrolysed remnants were spun down (10,000 *g*, 15 min) and 150 μl 0.2% cetylpyridinium chloride (CPC) was added to 150 μl supernatant. After 2 hr incubation at 37° the precipitate was centrifuged (10,000 *g* for 15 min) at 37°. The supernatant was discarded and the pellet was washed twice with 0.5 ml 0.05% CPC. The pellet was solubilized for 2 hr in 0.5 ml solulyte at 60° and after addition of scintillation fluid the quantity of radioactivity was analyzed with a liquid scintillation counter. Appropriate corrections were made for channel overlap of ^{35}S-counts and ^{3}H-counts.

Effect of paracetamol on the glycosaminoglycan synthesis in vivo. Two hours after oral administration of 200 mg/kg paracetamol or tap water to male Wistar rats 1 μCi/g [^{35}S]sulfate (carrier free) was injected intravenously (five rats per group). Blood samples were taken by orbita-punction at 20 min and 1, 2, 3, 4 and 5 hr after injection of [^{35}S]sulfate. Patellae were dissected from the knee joints 5 hr after administration of radiolabeled sulfate. Patellae were processed for determination of the [^{35}S]sulfate content as described above.

Total inorganic sulfate in serum of the rats was quantitated as described above. To determine the percentage of the free inorganic sulfate form of [^{35}S] in the serum of the rats, inorganic sulfate was precipitated with $BaCl_2$. To 50 μl of serum 1 ml of trichloroacetic acid (5% w/v) was added. This mixture was allowed to stand at room temperature for 10 min and was subsequently centrifuged for 15 min (10,000 *g*). Five hundred microlitres of clear supernatant was added to 125 μl $BaCl_2$ reagent (20 g $BaCl_2 \cdot 2H_2O$ and 100 g Dextran in 1 l distilled water). The precipitate was spun down (30 min, 10,000 *g*) after overnight incubation at 4°. The radioactivity in the pellet and supernatant was assayed by liquid scintillation counting. At whatever time, in the serum of control rats always more than 93% of total serum [^{35}S] was precipitated. In the serum of the paracetamol treated rats 50–60% of the total [^{35}S] was precipitable.

Characterization of glycosaminoglycans synthesized in vivo. Tap water or 200 mg/kg paracetamol was orally administrated to male Wistar rats (two rats per group) and after 2 hr a physiological saline solution with 1 μCi/g [^{35}S]sulfate and 8 μCi/g [^{3}H]glucosamine was injected intravenously. Five hours after injection of radiolabel, the patellae were dissected from the knee joints and articular cartilage was isolated as described above. After papain digestion and spinning down of the undigested remnants the supernatants were dialysed for 72 hr against tap water. This was followed by lyophilization and redissolving of the glycosaminoglycans in the elution buffers used during chromatography.

Glycosaminoglycans of individual patellae were applied on a DEAE–Trisacryl anion exchange column (1 × 15 cm) equilibrated with 200 ml 0.2 M NaCl in 1 mM HCl. A linear gradient of 0.2–2.0 M NaCl in 1 mM HCl was used to elute the glycosaminoglycans from the column. After each run the column was washed with 100 ml 0.2 M NaCl in 1 mM HCl. The flow was 20 ml/hr and fractions of 2 ml were collected. The fractions were assayed for radioactive labelled glycosaminoglycans by liquid scintillation counting.

Glycosaminoglycans of individual patellae were also applied on a Sepharose CL 6B gel chromatography column (100 × 0.7 cm). An 0.1 M ammonium acetate buffer (pH 5.0) was used as eluent. The flow was 15 ml/hr and fractions of 1.25 ml were collected and assayed for radioactive labeled glycosaminoglycans. The void volume (V_0) was determined by dextran blue ($M_r 2 \times 10^6$) and the total volume (V_t) by Na_2[^{35}S]SO_4.

Statistical analysis. Statistical analysis of data was performed by the two-tailed Student *t*-test. A P-value <0.05 was considered significant.

RESULTS

Decrease of serum sulfate level by paracetamol

Oral administration of 200 mg/kg paracetamol to Wistar rats led to a significant reduction of the inorganic sulfate concentration in serum (Fig. 1). Five

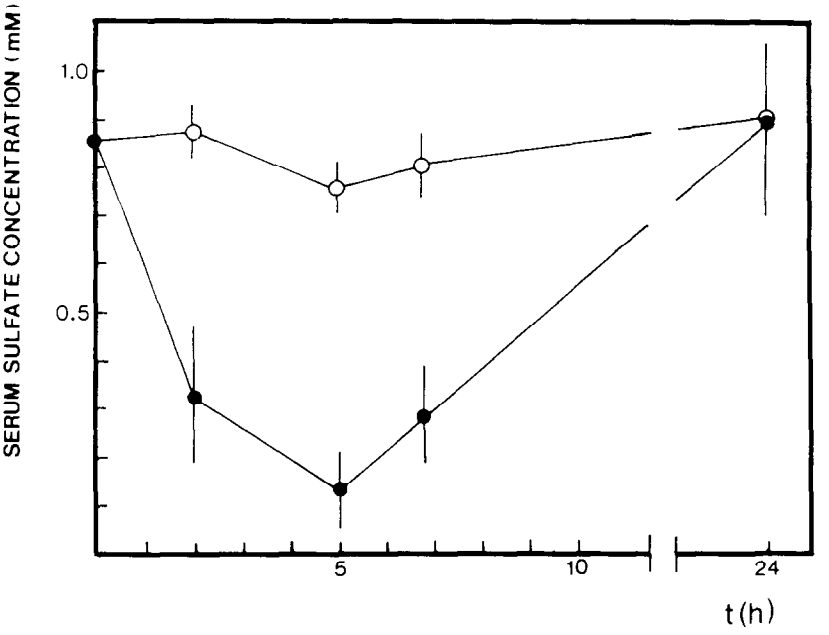

Fig. 1. Effect of the oral administration of 200 mg/kg paracetamol on the serum sulfate concentration of male Wistar rats. Control rats received a comparable volume of tap water. The results are expressed as the mean ± SD of five rats (●, paracetamol treated rats; ○, control rats).

hours after paracetamol administration the serum sulfate concentration was decreased by more than 80% after 7 hr the sulfate depletion was still 66%.

Effect of paracetamol and salicylate on [^{35}S]*sulfate labeled glycosaminoglycan synthesis* in vitro

In-vitro incubation of anatomically intact rat patellae with sodium salicylate resulted in a significantly decreased incorporation of [^{35}S]sulfate already at a concentration of 1 mM (Fig. 2). Paracetamol had up to a concentration of 5 mM, the highest concentration tested, no effect on incorporation of radiolabeled sulfate (oral administration of 200 mg/kg will result in a peak serum level of approximately 0.2–0.3 mM [15]). The incubations were carried out in the absence of serum, so the added drug concentrations were equivalent with the free drug concentrations in the incubation media.

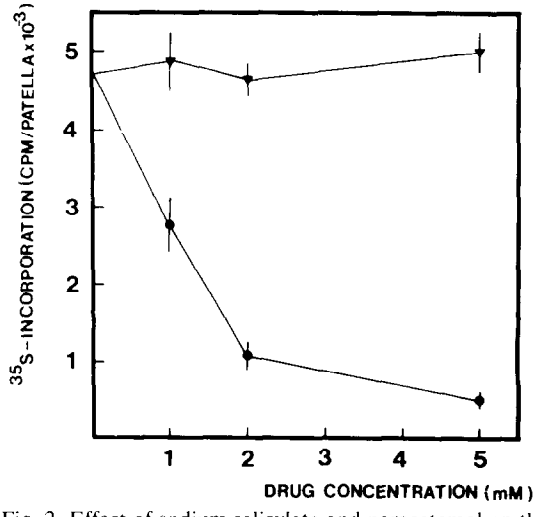

Fig. 2. Effect of sodium salicylate and paracetamol on the incorporation of [^{35}S]sulfate in anatomically intact articular cartilage of rat patellae *in vitro*. Patellae were incubated for 4 hr in medium containing sodium salicylate or paracetamol. For the last 2 hr the patellae were incubated in the presence of 20 μCi [^{35}S]sulfate. The results are expressed as the mean ± SD of five patellae (●, salicylate; ▼, paracetamol).

Effect of paracetamol on the degradation of [^{35}S]*sulfate labeled glycosaminoglycans*

Paracetamol had a slight but significant inhibitory effect on the catabolism of radiolabeled glycosaminoglycans *in vitro* (Table 1). All three paracetamol concentrations tested had a significant suppressing effect on the breakdown of radiolabeled glycosaminoglycans but a dose response was not observed.

Table 1. The effect of paracetamol on the degradation of [^{35}S]sulfate labeled glycosaminoglycans *in vitro*

Paracetamol (mM)	Chase (hr)	[^{35}S]sulfate content of patellae (cpm)
—	0	19,232 ± 3407
0	16	10,148 ± 878
1	16	13,040 ± 1153
2	16	13,864 ± 2768
5	16	11,777 ± 1427

Expressed are the mean values ± SD of at least five patellae. A 2-hr labeling period was followed by a 16-hr chase period in culture medium without radiolabel.

Effect of the sulfate concentration in medium on glycosaminoglycan synthesis

The incorporation of sulfate and glucosamine in the glycosaminoglycans of patellar cartilage incubated in medium with various sulfate concentrations is shown in Fig. 3. The incorporated quantity of sulfate and glucosamine was calculated by the incorporation of radiolabeled sulfate and glucosamine corrected for the specific activities of these precursors in the various experimental conditions. Incubation of patellae in medium containing less than 0.5 mM sulfate resulted in significantly reduced incorporation of sulfate in patellar cartilage glycosaminoglycans. The incorporation of glucosamine in patellar cartilage glycosaminoglycans was not diminished at sulfate concentrations below 0.5 mM. These results suggested the synthesis of undersulfated glycosaminoglycans at sulfate concentrations below 0.5 mM.

Effect of serum sulfate depletion on glycosaminoglycan synthesis in vivo

The *in-vitro* experiments described above show that a reduction of the sulfate concentration leads to a diminished sulfate incorporation and indicates the synthesis of undersulfated glycosaminoglycans at low sulfate concentrations. To answer the question if serum sulfate depletion leads to a diminished synthesis of glycosaminoglycans *in vivo*, rats were injected with radiolabeled sulfate 2 hr after oral administration of 200 mg/kg paracetamol. Patellae were dissected from the knee joints 5 hr after injection of radiolabel. The specific activity of [^{35}S]sulfate in the serum of the paracetamol treated and control rats in this 5-hr period is shown in Fig. 4. The area under the specific activity curve of the paracetamol treated rats was 1.52 greater than the area under the curve of the control rats. Moreover, the specific activity in the serum of the paracetamol treated rats decreased faster than the specific activity in the serum of the control rats.

The incorporation of radiolabel in the patellar cartilage of the paracetamol treated rats and the control rats is shown in Table 2. Incorporation of sulfate in the patellar cartilage of serum sulfate depleted rats was significantly diminished (66%). The paracetamol-induced serum sulfate depletion leads to a decreased synthesis of cartilage glycosaminoglycans.

Characterization of newly in vivo *synthesized glycosaminoglycans*

Our *in-vitro* experiments indicated the synthesis of undersulfated glycosaminoglycans at low sulfate concentrations. Therefore, *in-vivo* newly synthesized glycosaminoglycans were characterized by column chromatography to examine the presence of undersulfated glycosaminoglycans in the cartilage of the paracetamol treated rats. DEAE–Trisacryl anion exchange analysis of patellar glycosaminoglycans synthesized *in vivo* by paracetamol treated and control rats resulted in the chromatograms shown in Fig. 5. Both the chromatogram of the paracetamol treated and control rats showed the presence of one peak. The peak in both chromatograms eluted at a NaCl concentration of 0.7 M. In the chromatogram of the sulfate depleted rats (paracetamol treated) neither a shift of the sulfated glycosaminoglycan

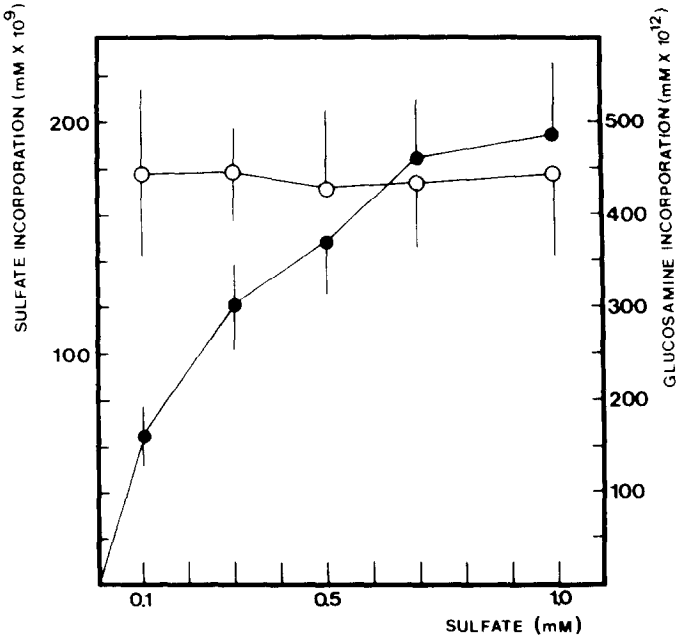

Fig. 3. Effect of sulfate concentration in the medium on incorporation of sulfate and glucosamine in anatomically intact articular cartilage of rat patellae. Patellae were incubated for 2 hr in BME-diploid medium with various sulfate concentrations. The results are expressed as the mean ± SD of at least five patellae (●, sulfate; ○, glucosamine).

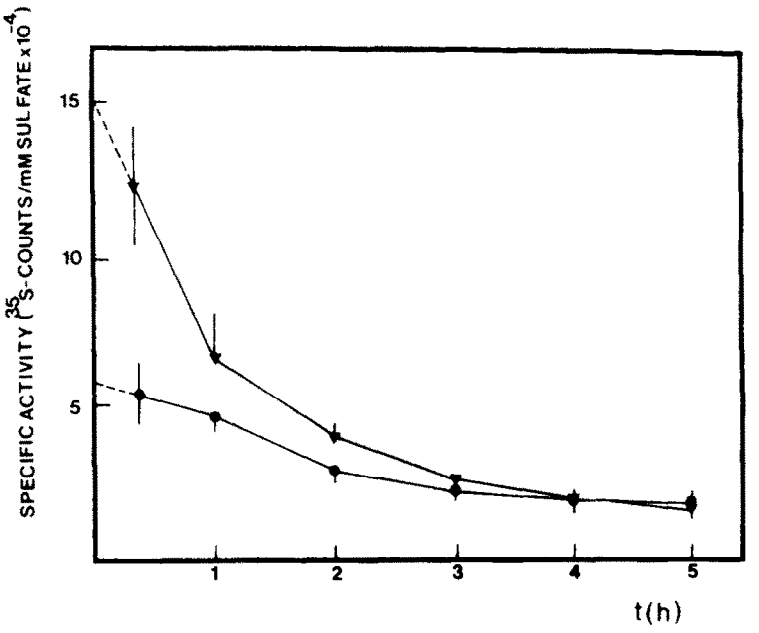

Fig. 4. The specific activity of free inorganic [^{35}S]sulfate in the serum of paracetamol treated and control rats. Paracetamol was orally administered 2 hr before injection of radiolabelled sulfate ($t = 0$). The results are expressed as the mean ± SD of five rats (▼, paracetamol-treated rats; ●, control rats).

peak nor the presence of a non-sulfated glycosaminoglycan peak was observed. Also the length of the newly synthesized glycosaminoglycan chains, as determined by gel chromatography with Sepharose CL 6B, was similar for the sulfate depleted and the control rats (Fig. 6). The K_{AV} of the glycosaminoglycan chains was 0.44.

DISCUSSION

The oral administration of 200 mg/kg paracetamol to male Wistar rats resulted in a significant reduction of the serum sulfate concentration. This serum sulfate depletion led to a diminished incorporation of [^{35}S]sulfate in patellar cartilage indicating a decreased glycosaminoglycan synthesis. Also incubation of rat patellae in medium containing less than 0.5 mM sulfate reduced the incorporation of sulfate. However, the incorporation of glucosamine was unaltered in these experiments at low sulfate concentrations in the medium. Characterization of patellar glycosaminoglycans synthesized *in vivo* could not confirm the production of undersulfated glycosaminoglycans as suggested by these results. The use of sulfate decreasing drugs can lead to a diminished glycosaminoglycan synthesis but will not alter the biochemical nature of these glycosaminoglycans, at least in short term.

In earlier studies we reported the inhibitory effect of salicylate induced sulfate depletion on glycosaminoglycan synthesis in mice [13, 14]. Salicylate induced an elevated excretion of sulfate by the kidneys [13, 14]. Incubation of murine cartilage in the presence of 5 mM salicylate inhibited sulfate incorporation 46% [13] while the incorporation in rat cartilage was inhibited by more than 90% (Fig. 2). Rat chondrocytes are more vulnerable to salicylate than murine chondrocytes.

The potential direct effects of salicylate on the metabolism of chondrocytes makes this drug less suitable for *in-vivo* studies on the effects of sulfate depletion on glycosaminoglycan synthesis [8]. Phe-

Table 2. Incorporation of [^{35}S]sulfate in patellar cartilage of paracetamol treated rats and control rats

	[^{35}S]sulfate incorporation in patellar cartilage		
	Non-corrected value (cpm)	Corrected value (cpm)	Percentage of control
Paracetamol-treated rats	6285 ± 692	4135 ± 455	66
Control rats	6051 ± 550	6051 ± 550	100

Expressed are mean values ± SD of the patellae of five rats. The values in the second column are corrected for the difference between the specific activity in the serum of the paracetamol treated rats and in the serum of the control rats (Fig. 4, area under the curve ratio is 1.52).

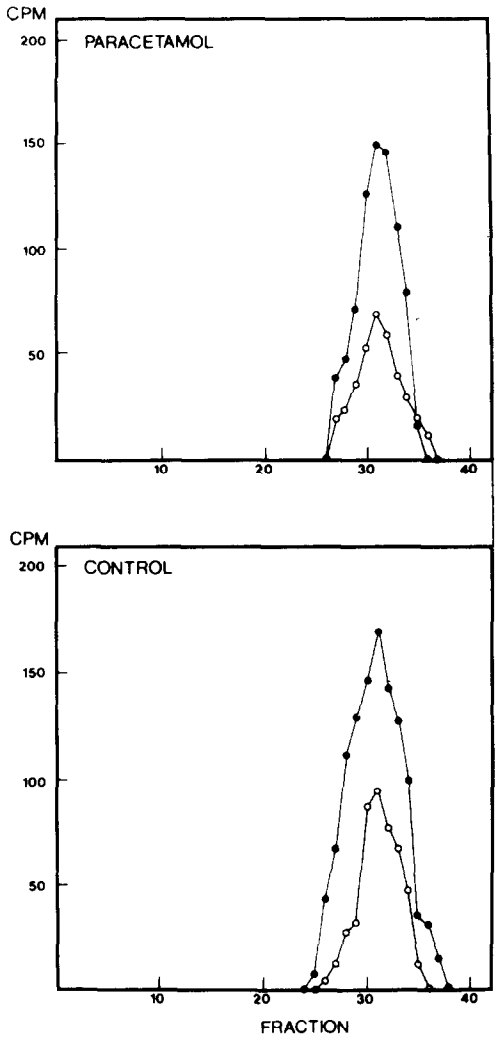

Fig. 5. DEAE–Trisacryl anion exchange chromatogram of newly synthesized patellar cartilage glycosaminoglycans from paracetamol-treated and control rats. Glycosaminoglycans were eluted with a NaCl gradient (0.2–2.0 M NaCl in 1 mM HCl). Fractions of 2 ml were collected and radioactivity was determined by liquid scintillation counting (●, [^{35}S]-counts; ○, [^{3}H]-counts).

nolic compounds like paracetamol and salicylamide are known to decrease the inorganic serum sulfate level by conjugation with sulfate [15, 20, 21]. Morris and Levy reported a significant reduction of the serum sulfate concentration in healthy human volunteers after a single dose of 1.5 g paracetamol [22].

Paracetamol (200 mg/kg) decreased the serum sulfate level in male Wistar rats significantly. The oral administration of this dose of paracetamol to male Wistar rats will result in a maximal serum paracetamol concentration of 0.2–0.3 mM, 1 hr after administration [15]. Paracetamol had no effect on the glycosaminoglycan synthesis up to the highest concentration tested (5 mM) while the glycosaminoglycan synthesis was already significantly inhibited by 1 mM salicylate (Fig. 2). Paracetamol had, *in vitro*, a slight inhibitory effect on the degradation of [^{35}S]sulfate labeled glycosaminoglycans. Paracetamol is a suitable drug to study the effect of sulfate depletion on glycsaminoglycan synthesis *in vivo* in the rat.

This study showed that low sulfate concentrations resulted both *in vitro* and *in vivo* in a diminished incorporation of sulfate in anatomically intact articular cartilage of the rat. Similar *in-vitro* results were found in experiments of Maroudas and Evans with slices of human and bovine cartilage and by Bayliss *et al.* for rabbit annulus cartilage [23, 24]. Also *in-vitro* incubation of anatomically intact patellar cartilage of mice in medium with less than 0.5 mM sulfate led to a diminished sulfate incorporation [13].

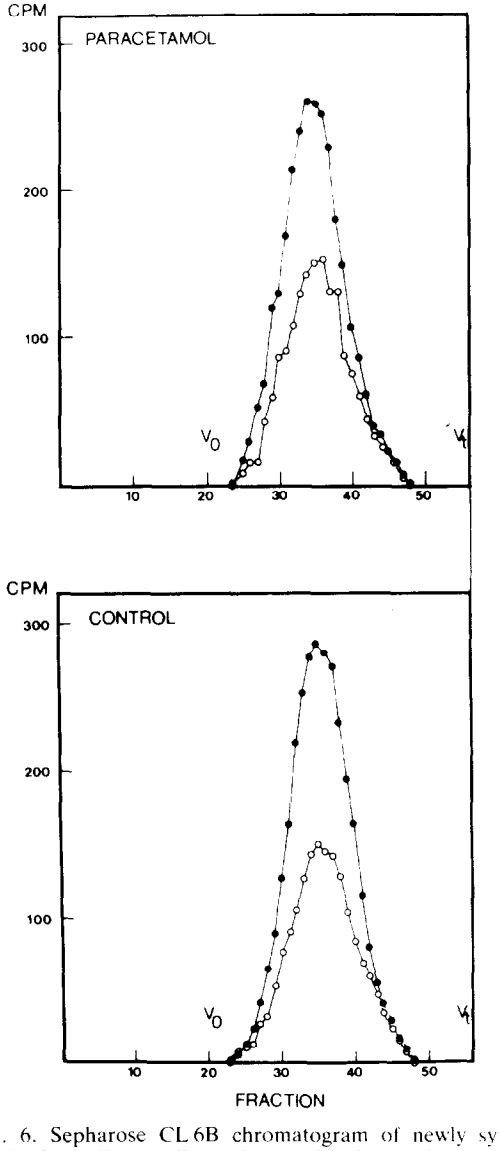

Fig. 6. Sepharose CL 6B chromatogram of newly synthesized patellar cartilage glycosaminoglycans from paracetamol treated and control rats. Glycosaminoglycans were eluted with an ammonium acetate buffer (0.1 M, pH 5.0). Fractions of 1.25 ml were collected and radioactivity was assayed by liquid scintillation counting (●, [^{35}S]-counts; ○, [^{3}H]-counts).

A decreased sulfate incorporation in cartilage is considered to be a reflection of a diminished glycosaminoglycan synthesis. However, the decreased sulfate incorporation in our *in-vitro* experiments did not show a concomitant decrease of glucosamine incorporation, a precursor of the glycosaminoglycan backbone. This suggested the synthesis of undersulfated glycosaminoglycans. Seegmiller *et al.* also found a decreased incorporation of sulfate and an unaltered incorporation of glucosamine after treatment of cartilage epiphyses from chick embryos with 6-aminonicotinamide [25]. Characterization with anion exchange chromatography of patellar cartilage glycosaminoglycans synthesized *in vivo* at decreased sulfate concentrations could not confirm the synthesis of undersulfated glycosaminoglycans (Fig. 5). Incubation of murine patellae *in vitro* at low sulfate concentrations (<0.5 mM) also resulted in decreased sulfate and unaltered glucosamine incorporation. The glycosaminoglycans synthesized at a high sulfate concentration (1 mM) were similar to those synthesized at a low sulfate concentration (0.1 mM) (manuscript submitted).

The unaltered incorporation of glucosamine in the presence of a decreased glycosaminoglycan synthesis could be the result of changes in the intracellular specific activity of [^{3}H]glucosamine. We postulate the hypothesis that at a high rate of glycosaminoglycan synthesis the demand for glucosamine will be high and the glucose component of glucosamine will be derived from the intracellular glycogen pool in the chondrocytes. A decreased synthesis of glycosaminoglycans as a consequence of sulfate shortage or glycosaminoglycan synthesis inhibiting compounds will result in a reduced degradation of glycogen to glucose. The reduced intracellular supply of glucose and consequently of unlabeled glucosamine will result in an increased specific activity of [^{3}H]glycosamine. This could be an explanation for the apparent unaltered incorporation of glucosamine at low sulfate concentrations.

In this study we demonstrate that reduction of the serum sulfate concentration can lead to inhibition of glycosaminoglycan synthesis *in vivo*. The biochemical quantities of the glycosaminoglycans synthesized at low sulfate concentration were similar with those synthesized at physiological sulfate concentrations. Conjugation with sulfate is a common physiological way of drug detoxification [15]. Drugs can also effect the renal excretion of sulfate [13, 14]. Both mechanisms can lead to sulfate depletion and consequently to inhibition of glycosaminoglycan synthesis. The glycosaminoglycan synthesis in man will be extremely sensitive for sulfate depletion because man has a very low serum sulfate level (0.3–0.4 mM) and sulfate concentrations just below 0.3 mM do already result in a diminished synthesis of glycosaminoglycans in human articular cartilage [22, 23]. The use of potential serum sulfate decreasing drugs might have deleterious effects on cartilage, especially when used for prolonged periods. This argues for a reconsideration of the use of potential sulfate decreasing drugs in patients with rheumatoid arthritis or osteoarthritis who already have a disturbed cartilage metabolism.

REFERENCES

1. Whitehouse MW and Bostrom H, Studies on the action of some anti-inflammatory agents in inhibiting the biosynthesis of mucopolysaccharidesulphates. *Biochem Pharmacol* **7**: 135–150, 1961.
2. Bostrom H, Berntsen K and Whitehouse MW, Biochemical properties of antiinflammatory drugs—I. Some effects on sulphate-^{35}S metabolism *in vivo*. *Biochem Pharmacol* **13**: 413–420, 1964.
3. Vidal y Plana RR, Bizzarri D and Rovati LL, Articular cartilage pharmacology—I. In vitro studies on glucosamine and non steroidal antiinflammatory drugs. *Pharmacol Res Commun* **10**: 557–569, 1978.
4. Palmoski MJ and Brandt KD, Effects of some nonsteroidal antiinflammatory drugs on proteoglycan metabolism and organization in canine articular cartilage. *Arthritis Rheum* **23**: 1010–1020, 1980.
5. Brandt KD and Palmoski MJ, Effects of salicylates and other nonsteroidal anti-inflammatory drugs on articular cartilage. *Am J Med* **77(1A)**: 65–69, 1984.
6. Brandt KD and Slowman-Kovacs S, Nonsteroidal antiinflammatory drugs in treatment of osteoarthritis. *Clin Orthop Rel Res* **213**: 84–91, 1986.
7. McKenzie LS, Horsburg BA, Gosh P and Taylor TKF, Effect of anti-inflammatory drugs on sulphated glycosaminoglycan synthesis in aged human articular cartilage. *Ann Rheum Dis* **35**: 487–497, 1976.
8. Palmoski MJ and Brandt KD, Effect of salicylate on proteoglycan metabolism in normal canine articular cartilage *in vitro*. *Arthritis Rheum* **22**: 746–754, 1979.
9. Palmoski MJ, Coyler RA and Brandt KD, Marked suppression by salicylate of the augmented proteoglycan synthesis in osteoarthritic cartilage. *Arthritis Rheum* **23**: 83–91, 1980.
10. Palmoski MJ and Brandt KD, Aspirin aggravates the degeneration of canine joint cartilage caused by immobilization. *Arthritis Rheum* **25**: 1333–1342, 1982.
11. Palmoski MJ and Brandt KD, In vivo effect of aspirin on canine osteoarthritic cartilage. *Arthritis Rheum* **26**: 994–1001, 1983.
12. Palmoski MJ and Brandt KD, Partial reversal by beta-D-xyloside of salicylate-induced inhibition of glycosaminoglycan synthesis in articular cartilage. *Arthritis Rheum* **25**: 1084–1093, 1982.
13. de Vries BJ, van den Berg WB and van de Putte, LBA, Salicylate induced depletion of endogenous inorganic sulfate: potential role in the suppression of sulfated glycosaminglycan synthesis in murine articular cartilage. *Arthritis Rheum* **28**: 922–929, 1985.
14. de Vries BJ, van den Berg WB, Vitters E and van de Putte, LBA, The effect of salicylate on anatomically intact articulatar cartilage is influenced by sulfate and serum in the culture medium. *J Rheumatol* **13**: 686–693, 1986.
15. Krijgsheld KR, Scholtens E and Mulder GJ, An evaluation of methods to decrease the availability of inorganic sulphate for sulphate conjugation in the rat *in vivo*. *Biochem Pharmacol* **30**: 1973–1979, 1981.
16. Dixon MF, Dixon B, Aparilo SR and Loney DP, Experimental paracetamol-induced hepatic necrosis: a light- and electron-microscope, and histochemical study. *J Path* **116**: 17–29, 1975.
17. Jollow DJ, Thorgeirsson SS, Potter WZ, Hashimoto M and Mitchell JR, Acetaminophen-induced hepatic necrosis—VI. Metabolic disposition of toxic and nontoxic doses of acetaminophen. *Pharmacology* **12**: 251–271, 1974.
18. Dogson KS and Spencer B, Studies on sulphatases—5. The determination of inorganic sulphate in the study of sulphatases. *Biochem J* **55**: 436–440, 1953.
19. de Vries BJ, Vitters E, van den Berg WB, Schram D and van de Putte LBA, Determination of small

quantities of sulfate (0–12 nmol) in serum, urine, and cartilage of the mouse. *Anal Biochem* **163**: 408–417, 1987.
20. McCarry PC and Roe DA, Development of sulfur depletion in pregnant and fetal rats: interaction of protein restriction and indole or salicylamide. *J Nutr* **103**: 1279–1290, 1973.
21. Halstead PK and Roe DA, Effect of salicylamide on skeletal glycosaminoglycan sulfation and calcification in fetal rat limbs. *Drug Nutr Interact* **1**: 75–86, 1981.
22. Morris ME and Levy G, Serum concentration and renal excretion by normal adults of inorganic sulfate after acetaminophen, ascorbic acid, or sodium sulfate. *Clin Pharmacol Ther* **33**: 529–536, 1983.
23. Maroudas A and Evans H, Sulphate diffusion and incorporation into human articular cartilage. *Biochim Biophys Acta* **338**: 265–279, 1974.
24. Baylis MT, Urban JPG, Johnston B and Holme S, In vitro method for measuring synthesis rates in the intervertebral disc. *J Orthop Res* **4**: 10–17, 1986.
25. Seegmiller RE, Horwitz AL and Dorfman A, Alterations of chondroitin sulfate synthesized by chick embryo cartilage cultured in the presence of 6-aminonicotinamide. *J Embryol Exp Morph* **59**: 207–216, 1980.