Control of proteoglycan biosynthesis

Further studies on the effect of serum on cultured bovine articular cartilage

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Proteoglycan synthesis in explant cultures of adult bovine articular cartilage is stimulated in a dose-dependent manner when the tissue is cultured in the presence of foetal-calf serum. The stimulation of proteoglycan synthesis is paralleled by a similar increase in DNA synthesis; however, when DNA synthesis is inhibited by hydroxyurea the stimulation of proteoglycan synthesis by serum remains essentially the same. The apparent half-life of the pool of proteoglycan core protein precursor was measured in freshly isolated tissue as well as in tissue cultured for 7 days in the presence and in the absence of foetal-calf serum; under all conditions the half-life was the same, suggesting that this value is independent of the net rate of proteoglycan synthesis. In the presence of actinomycin D, an inhibitor of RNA synthesis, there was a difference in the apparent half-life of the available pool of mRNA coding for proteoglycan core protein: 8.5 h for tissue maintained in the presence of serum and 3.8 h for tissue cultured in the absence of serum. It is suggested that proteoglycan synthesis is stimulated by serum factors at the level of DNA-dependent RNA synthesis. Concomitant with an increase in the rate of proteoglycan synthesis induced by the presence of serum in the culture medium, an increase in the concentrations of several glycosyltransferases involved in chondroitin sulphate synthesis was also observed.

INTRODUCTION

Cartilage is a connective tissue composed predominantly of type II collagen and cartilage-specific chondroitin sulphate proteoglycan. Under normal physiological conditions, the amount of proteoglycan within the extracellular matrix is maintained by the constituent chondrocytes at a relatively constant level. Proteoglycans are continuously being synthesized, incorporated into the extracellular matrix, and then actively lost from the tissue (Hascall *et al.*, 1983a,b; Campbell *et al.*, 1984). Changes in the rates of synthesis or catabolism will lead to changes in the amount of proteoglycan within the extracellular matrix, and this ultimately alters the mechanical properties of the tissue (Kempson *et al.*, 1976). Regulation of proteoglycan homeostasis within the matrix of articular cartilage is therefore important for maintenance of the tissue's normal physiological function.

The biosynthesis of a cartilage-specific proteoglycan molecule is complex. Synthesis of the proteoglycan core protein (M_r approx. 2.0×10^5) within the rough endoplasmic reticulum is followed by the addition of many complex carbohydrate chains, including small *N*-linked and *O*-linked oligosaccharides and up to 150 glycosaminoglycan chains (chondroitin sulphate and keratan sulphate), resulting in a molecule of M_r approx. 2×10^6 – 4×10^6 (for reviews see Rodén, 1980; Hascall & Hascall, 1981). The control of cartilage proteoglycan biosynthesis remains unclear, but is certainly influenced by a variety of factors, including the concentrations of matrix components, mechanical stress on the tissue and the concentrations and nature of circulating hormones and growth factors (for review see Handley *et al.*, 1985).

In addition, various intrinsic control mechanisms have been postulated that might regulate the rate of proteoglycan synthesis and the type of molecule being synthesized (Dorfman, 1981; McQuillan *et al.*, 1984, 1986).

We have previously shown (Hascall *et al.*, 1983a) that proteoglycan synthesis by slices of bovine articular cartilage in culture is stimulated in the presence of foetal-calf serum. In the absence of serum, there is a steady decline in the rate of synthesis of proteoglycan coupled with a loss of proteoglycan from the tissue. Nevertheless, in tissue maintained in the absence of serum for several days the rate of proteoglycan synthesis is increased to the higher values over 2–3 days after transfer of the tissue to medium containing serum. Proteoglycans synthesized by cultures in the presence or in the absence of serum are of a size typical for this tissue, and the stimulation therefore represents a co-ordinated increase in the total amount of proteoglycan being processed per unit time. In the present study, the effect of serum on proteoglycan biosynthesis by explant cultures of articular cartilage has been characterized further. We describe experiments whereby the intracellular pools of both proteoglycan core protein precursor and the mRNA coding for core protein are compared for cultures maintained in the presence and in the absence of serum.

EXPERIMENTAL

Materials

Hydroxyurea was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other materials were

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obtained as previously described (Hascall *et al.*, 1983a; McQuillan *et al.*, 1984, 1986).

Cartilage cultures

Explant cultures of the articular cartilage from the metacarpal-phalangeal joints of 1–2-year-old steers were prepared as described by Hascall *et al.* (1983a). Tissue was maintained in batch culture for up to 6 days in either medium alone (Handley & Lowther, 1977) or medium supplemented with foetal-calf serum, as indicated in the Results section. After the appropriate time in batch culture, the tissue was distributed into individual portions of 50–100 mg wet wt. before further treatment.

Incubation of cultures with [³⁵S]sulphate

For each experiment, a sufficient amount of labelling medium was prepared so that each culture was incubated in an identical volume. Before labelling, each culture was preincubated in fresh medium for 1 h, and this medium was then replaced with 2 ml of labelling medium containing 20–30 μ Ci of [³⁵S]sulphate/ml. Labelling of tissue in loosely capped culture vials was performed at 37 °C in a shaker bath for periods ranging from 20 min to 2 h.

Extraction procedures

Incubation of cultures with [³⁵S]sulphate was terminated in one of three ways. In some experiments, the tissue samples were first extracted at 4 °C for 2–4 days with 2 ml of 4 M-guanidinium chloride/0.5 M-sodium acetate buffer, pH 5.8, in the presence of proteinase inhibitors; this extracted more than 70% of ³⁵S-labelled proteoglycans (Hascall & Kimura, 1982). After removal of guanidinium chloride extracts, tissue residues were treated for an additional 1–2 days at room temperature with 2 ml of 0.5 M-NaOH, which extracted all of the remaining macromolecular radioactivity from the tissue (Hascall & Kimura, 1982). In some experiments, tissue samples were treated directly with 2 ml of 0.5 M-NaOH to extract the total glycosaminoglycan pool in one step. In other experiments, the total glycosaminoglycan pool was recovered by the direct addition of 0.2 ml of 5.0 M-NaOH to the medium plus tissue. Samples (usually 0.25 ml) of extracts were fractionated on Sephadex G-25 (PD-10) columns (Kimura *et al.*, 1979).

Analytical gel chromatography

A column of Sepharose CL-2B (0.8 cm $\times$ 100 cm) was eluted with 4 M-guanidinium chloride/0.2 M-Na₂SO₄/0.5% (v/v) Triton X-100/0.1 M-sodium acetate buffer, pH 6.0. Fractions (1.4 ml) were collected and assayed for radioactivity (Hascall *et al.*, 1983a).

Incorporation of [³H]thymidine into DNA

Cultures were preincubated for 1 h in fresh medium before incubation for 2 h in 2 ml of medium containing 10 μ Ci of [³H]thymidine/ml. Labelling was terminated by removal of the medium followed by digestion of the tissue with papain (Handley & Lowther, 1977). When digestion was complete, the DNA was precipitated by the addition of an equal volume of ice-cold 20% (w/v) trichloroacetic acid. The resultant suspension was passed over glass-microfibre filters (Whatman, 2.5 cm GF/A) under vacuum followed by extensive washing with ice-cold 5% (w/v) trichloroacetic acid containing 20 mM-thymidine. The DNA bound to the filters was fixed by

further washing with ice-cold ethanol. The glass-microfibre filters were dried at 100 °C for 30 min, suspended in a scintillation cocktail described by Fox (1968), and the rate of incorporation of [³H]thymidine into acid-insoluble material was determined.

Enzyme assays

The following glycosyltransferase activities were assayed as previously described (McQuillan *et al.*, 1984; Robinson & Robinson, 1985): galactosyltransferase I (UDP-galactose: β -D-xylopyranoside galactosyltransferase); N-acetylgalactosaminyltransferase (UDP-N-acetylgalactosamine:chondroitin N-acetylgalactosaminyltransferase); glucuronosyltransferase II (UDP-D-glucuronic acid:chondroitin D-glucuronosyltransferase); galactosyltransferase II (UDP-galactose:D-galactoside galactosyltransferase); UDP-galactose: β -N-acetylglucosaminide galactosyltransferase. The assay of explant cultures of articular cartilage for enzyme activity has been described previously (McQuillan *et al.*, 1984).

RESULTS

Stimulation of proteoglycan synthesis by serum and its relation to cell division

The effect of different serum concentrations on the rate of proteoglycan synthesis was examined. Cultures were maintained for 5 or 10 days in medium containing proportions of foetal-calf serum of 0 to 20% (v/v). Tissue was incubated with [³⁵S]sulphate for 2 h, and the labelled glycosaminoglycans were isolated and quantified. The rate of proteoglycan synthesis by cultures maintained for 10 days in the absence of serum was 52% of that measured for similar cultures labelled on day 5 (Fig. 1), suggesting that there had been a progressive slow decline in the rate of proteoglycan synthesis by tissue maintained in medium alone over the entire period of culture. Both day-5 and day-10 (Fig. 1) cultures exhibited similar dose-response curves, which demonstrate a correlation between an increase in the rate of proteoglycan synthesis and the concentration of serum in the medium.

The rate of synthesis at higher serum concentrations was essentially the same for day-5 and day-10 cultures, consistent with our previous observation that the metabolism of tissue maintained with serum was at a steady state during the period of culture.

Samples of 4 M-guanidinium chloride extracts of cultures maintained in the presence of 0%, 5%, 10% and 20% (v/v) foetal-calf serum for 10 days (Fig. 1, points a–d) were fractionated on a column of Sepharose CL-2B. The profiles obtained were similar (Fig. 2), with the major proteoglycan component being eluted at essentially the same position irrespective of the concentration of foetal-calf serum in the medium ($K_{av} \approx 0.22$). The tissue concentration of the minor proteoglycan (hatched area) did not change in the course of this experiment and represented 5–7% of the total labelled pool of proteoglycans in the tissue. Although the rate of proteoglycan synthesis varied quite markedly with increasing serum concentration (Fig. 1), the hydrodynamic size of the proteoglycans synthesized under these steady-state conditions was unchanged.

Many growth factors in serum have been shown to have a high mitogenic potential for cells *in vitro* (for reviews see Baserga, 1981; Spencer, 1983). An increase in

Fig. 1. Effect of foetal-calf serum concentration on the rate of proteoglycan synthesis

Bovine articular cartilage was maintained in explant culture for 5 days (●) and 10 days (○) in medium supplemented with 0–20% (v/v) foetal-calf serum. Cultures were incubated with [^{35}S]sulphate for 2 h, and the ^{35}S -labelled macromolecules were isolated from the cartilage by sequential extraction with guanidinium chloride and NaOH, and these extracts were analysed by gel chromatography on PD-10 columns. The rates of [^{35}S]sulphate incorporation into total proteoglycans (i.e. sum of 4 M-guanidinium chloride and 0.5 M-NaOH extracts for each sample) are given. Each point represents the mean of duplicate determinations, with the deviation from the mean less than $\pm 8\%$ in all cases. The letters (a)–(d) indicate the samples used for analyses in Fig. 2.

the total cell population of the tissue could conceivably give rise to an increase in the measured rate of proteoglycan synthesis without the requirement for increased cellular metabolism. Hydroxyurea is an inhibitor of cell division and cell proliferation in many tissues (Sung, 1969), although the exact mechanism of its action is unclear. At a concentration of 5 mM, hydroxyurea induces an immediate inhibition of DNA synthesis with no apparent effect on either RNA or protein synthesis (Gallwitz & Mueller, 1969). Experiments showed that 5 mM-hydroxyurea induced total inhibition of DNA synthesis by cultured cartilage with no marked inhibition of proteoglycan synthesis (results not shown). In the experiments described below, 5 mM-hydroxyurea was used to inhibit DNA synthesis.

Articular cartilage from 1–2-year-old steer was maintained in the presence of medium alone for 6 days. The tissue was then distributed into vials and maintained for

Fig. 2. Elution from Sepharose CL-2B of proteoglycans synthesized by cultures of bovine articular cartilage maintained in the presence of different concentrations of foetal-calf serum

Samples of the 4 M-guanidinium chloride extracts indicated by the letters (a)–(d) in Fig. 1 were eluted from a column of Sepharose CL-2B under dissociative conditions. Extracts were obtained from cultures maintained for 10 days in the presence of (a) medium alone, (b) 5% (v/v) foetal-calf serum, (c) 10% (v/v) foetal-calf serum and (d) 20% (v/v) foetal-calf serum. The percentage values indicate the proportion of total macromolecular label in the hatched peaks. V_0 , Void volume; V_t , total volume.

a further 1–4 days in (a) medium alone, (b) medium containing 20% (v/v) foetal-calf serum or (c) medium containing 20% (v/v) foetal-calf serum and 5 mM-hydroxyurea. On the days indicated in Fig. 3, cultures were incubated with either [^{35}S]sulphate or [^3H]thymidine for 2 h. The stimulation of DNA synthesis by foetal-calf serum (● symbols) followed a similar time course to that of proteoglycan synthesis (○ symbols), but when DNA synthesis was abolished by hydroxyurea (■ symbols) there was only a slight inhibition of proteoglycan synthesis (□ symbols). Foetal-calf serum increased the rate of DNA synthesis, but this stimulation was not essential for the stimulation of proteoglycan synthesis. It is conceivable that foetal-calf serum induces a progression of cells from G_0 or G_1 phase to S phase, which could be consistent with the notion that the increase in proteoglycan synthesis is independent of cell division, yet dependent on the state of chondrocytes in their cell cycle. These observations are also consistent with results reported by Benya *et al.* (1984), in which an increase in DNA synthesis was attributed to cellular outgrowth on the surface of the explant cultures. Irrespective of the

Fig. 3. Effect of hydroxyurea on the rates of proteoglycan and DNA synthesis in bovine articular cartilage

Bovine articular cartilage was maintained in explant culture in medium alone for 6 days. Cultures were further maintained for up to 4 days in either medium alone (Δ and $\blacktriangle$), medium containing 20% (v/v) foetal-calf serum ($\circ$ and $\bullet$) or medium containing 20% (v/v) foetal-calf serum plus 5 mM hydroxyurea ($\square$ and $\blacksquare$). On the days indicated, cultures were incubated for 2 h with either [35 S]sulphate or [3 H]thymidine and the rates of synthesis of proteoglycan (Δ , $\circ$ and $\square$) and DNA ($\blacktriangle$, $\bullet$ and $\blacksquare$) were determined (see the Experimental section). The vertical bars indicate the average range of duplicates for each curve.

mechanisms involved, it is evident that DNA replication is not essential for an increased rate of proteoglycan synthesis.

Effect of foetal-calf serum on the half-life of the proteoglycan core protein

The presence or the absence of foetal-calf serum results in a stimulation or depression of the rate of proteoglycan synthesis by bovine articular cartilage respectively. These different rates of proteoglycan synthesis may reflect differences in the size of the pool of core protein present in the tissue under different conditions of culture. With the use of procedures described previously (McQuillan *et al.*, 1984) the half-lives of the pools of core protein for proteoglycan were determined. Cultures were prepared from the metacarpal-phalangeal articular cartilage of a 1–2-year-old steer and maintained in culture for up to 7 days, both in medium alone and in medium containing 20% (v/v) foetal-calf serum. On days 0 and 7 of culture, tissue was incubated with 0.4 mM-cycloheximide for up to 120 min. At 20 min intervals following the addition of cycloheximide, cultures were incubated with [35 S]sulphate for 20 min and the rate of proteoglycan synthesis was

determined for each interval (Fig. 4). The rate of proteoglycan synthesis at time zero by tissue maintained for 7 days with serum (Fig. 4, $\circ$ symbols) was about 3 times that for tissue maintained in serum-free medium for the same period of time (Fig. 4, $\square$ symbols). Compared with the rate of proteoglycan synthesis on day 0 (Fig. 4, $\bullet$ symbols), tissue cultured in the presence of serum was stimulated by 30%.

The data shown in Fig. 4 fit very closely to a first-order equation describing the depletion of proteoglycan core protein from an intracellular pool:

$$y = C \cdot e^{-at}$$

where y is the rate of proteoglycan synthesis ([35 S]sulphate incorporation) at any time t , C is the rate of synthesis measured at zero time ($t = 0$), and a is the exponential decay constant derived from semi-logarithmic plots of experimentally determined values for y versus t (Fig. 4 inset). Irrespective of the time in culture in the presence or in the absence of serum, the gradient of the lines obtained in all three cases (and therefore the value of a) was the same. Since the pool size of the core protein precursor (P) can be described by the relationship

$$P = \int C \cdot e^{-at} \cdot dt = C/a$$

it follows that when the value of a is constant the size of the pool of core protein is directly proportional to the

Fig. 4. Effect of serum on the pool size of proteoglycan core protein precursor in bovine articular cartilage

Cultures of bovine articular cartilage were maintained for up to 6 days in the presence or in the absence of 20% (v/v) foetal-calf serum. On day 0 or day 6, individual cultures were exposed to 0.4 mM-cycloheximide for up to 120 min. At 20 min intervals, duplicate cultures were incubated with [35 S]sulphate for 20 min and the rate of glycosaminoglycan synthesis was determined for each interval. Curves are shown for day-0 cultures ($\bullet$) and for day-6 cultures maintained with ($\circ$) and without ($\square$) 20% (v/v) foetal-calf serum. The inset shows a semi-logarithmic plot of the experimental results (see the text for details). Each point represents the mean of duplicate determinations.

initial rate of synthesis. In the presence of serum the rate of synthesis is increased, and this can be explained by an increase in the amount of core protein precursor.

In addition to our previous observation that the half-life of the pool of core protein is a remarkably constant value in bovine articular cartilage (McQuillan *et al.*, 1984), these results further demonstrate that the rate of proteoglycan synthesis is most probably modulated at the level of proteoglycan-core-protein pool size.

Effect of serum on the half-life of the mRNA for proteoglycan core protein in bovine articular cartilage

The stimulation of proteoglycan synthesis by serum appeared to be manifest at a step before the processing of the proteoglycan core protein. In previous studies (McQuillan *et al.*, 1986) we have used inhibitors of RNA synthesis in order to assess the relation of the availability of mRNA to the rate of proteoglycan biosynthesis by explant cultures. In these previous studies we concluded that the pool of mRNA coding for the proteoglycan core protein in bovine explant cultures maintained with serum had a half-life of about 7 h. The possibility that the size or stability of the pool of mRNA for proteoglycan biosynthesis was influenced by serum factors was examined.

Tissue from the articular cartilage of a 1–2-year-old steer was maintained in the presence or in the absence of 20% (v/v) foetal-calf serum for 7 days. Cultures were exposed to actinomycin D (10 μ g/ml) for up to 24 h, and

at the times indicated in Fig. 5 cultures were incubated with [35 S]sulphate for 1 h and the rate of proteoglycan synthesis was determined for each interval. The rate of proteoglycan synthesis by cultures maintained in the presence of serum (Fig. 5, $\circ$ symbols) decreased exponentially after exposure of the cultures to actinomycin D, with a calculated half-life of 8.5 h (Fig. 5 inset). Tissue that had been maintained in the presence of medium alone showed a similar decay in proteoglycan synthesis (Fig. 5, $\square$ symbols), but with a calculated half-life of 3.8 h (Fig. 5 inset). These data suggest that the pool of mRNA in tissue maintained in the presence of serum may be about 2-fold greater than in tissue maintained in the absence of serum. This contrasts with the measured half-lives for the pool of core protein in tissue maintained under similar conditions, which were the same. Furthermore, the ratio of the initial rates of synthesis with serum to tissue maintained without serum (2.2:1) is remarkably similar to the ratio of the calculated mRNA half-lives (2.1:1). It seems likely that the initial rate of synthesis is determined by the size of the available pool of mRNA coding for proteoglycan core protein.

Effect of foetal-calf serum on glycosyltransferase activity in bovine articular cartilage

The effect of foetal-calf serum was further investigated by measuring the activity of some glycosyltransferases involved in the synthesis of chondroitin sulphate. Cultures were maintained for 5 days with or without 20% (v/v) foetal-calf serum. Some cultures were homogenized in the presence of a detergent to extract membrane-bound enzymes. The remaining cultures were switched either from medium alone to medium containing serum, or from medium containing serum to medium alone. At 3 days after the switch, these cultures were also homogenized and the enzymes extracted, as described above.

Two enzymes involved in synthesis of the polysaccharide-protein linkage oligosaccharide of chondroitin sulphate (galactosyltransferase I and galactosyltransferase II) and two enzymes involved in elongation of the chondroitin chain (*N*-acetylgalactosaminyltransferase and glucuronosyltransferase II) were assayed. In addition, the activity of a non-specific glycoprotein galactosyltransferase was determined.

After 5 days in culture, when essentially steady-state rates of proteoglycan synthesis are reached, the rate of synthesis of proteoglycans by cultures maintained in the absence of serum was 28% of the value observed for tissue maintained in the presence of foetal-calf serum (Table 1). Of the enzymes involved in chondroitin sulphate synthesis, the largest decrease in activity was observed for galactosyltransferase I: a 65% decrease compared with cultures maintained in the presence of serum.

It was further shown (Table 1) that the low enzyme concentrations apparent for cultures maintained in medium alone could be elevated by switching the cultures to medium containing serum. Thus, as reported for the effect of serum on the rate of proteoglycan synthesis (Hascall *et al.*, 1983a), the effect of foetal-calf serum was also reversible for chondroitin sulphate glycosyltransferase activity.

In addition to the chondroitin sulphate-specific glycosyltransferases, another enzyme activity was also

Fig. 5. Effect of serum on the half-life of proteoglycan-core-protein mRNA in bovine articular cartilage

Bovine articular cartilage was maintained for 7 days in the presence ($\circ$) and in the absence ($\square$) of 20% (v/v) foetal-calf serum. At zero time (on day 7) cultures were exposed to 10 μ g of actinomycin D/ml for up to 24 h. At the times shown, duplicate cultures were incubated with [35 S]sulphate for 1 h and the rate of proteoglycan synthesis for each interval was determined (see the Experimental section). The half-life of each curve was determined from semi-logarithmic plots of the experimental results (inset).

Table 1. Effect of serum on the activity of glycosyltransferases in bovine articular cartilage

Cultures of bovine cartilage were maintained with or without foetal-calf serum for 5 days. Other cultures were switched on day 5 either into medium with foetal-calf serum or into medium without serum and maintained a further 3 days. At the times shown, cultures were homogenized in enzyme buffer and assayed for glycosyltransferase activity. Identical cultures were incubated with [³⁵S]sulphate for proteoglycan synthesis. Each value represents the mean of two determinations ± deviation from the mean. Values in parentheses indicate the percentage of the value shown at the top of each column (i.e. tissue maintained for 5 days with serum designated 100% for each column). Abbreviations: Gal-T-I, galactosyltransferase I; Gal-T-II, galactosyltransferase II; GlcA-T-II, glucuronosyltransferase II; GalNAc-T, *N*-acetylgalactosaminyltransferase; GlcNAc-Gal-T, UDP-galactose: *N*-acetylglucosaminide galactosyltransferase; N.D., not determined.

Conditions of culture	[³⁵ S]sulphate incorporation (d.p.m./2 h per mg wet wt.)	10 ⁻² × Activity (nmol of substrate transferred/h per mg wet wt.)				
		Gal-T-I	Gal-T-II	GlcA-T-II	GalNAc-T	GlcNAc-Gal-T
5 days with serum	3374 ± 47	14.8 ± 0.2	13.6 ± 0.3	11.0 ± 0.5	7.5 ± 0.2	31.9 ± 2.1
5 days medium alone	946 ± 14 (28)	5.2 ± 0.3 (35)	7.9 ± 0.3 (58)	6.4 ± 0.2 (58)	8.7 ± 0.4 (116)	7.6 ± 0.4 (24)
5 days medium alone, 3 days with serum	3425 ± 105 (102)	16.9 ± 0.4 (114)	14.3 ± 0.3 (105)	N.D.	N.D.	19.9 ± 1.1 (62)
5 days with serum, 3 days medium alone	1150 ± 84 (34)	5.6 ± 0.2 (38)	7.5 ± 0.4 (55)	N.D.	N.D.	9.3 ± 0.6 (29)

assayed, namely UDP-galactose: *N*-acetylglucosaminide galactosyltransferase, which catalyses the transfer of galactose to *N*-acetylglucosamine residues in glycoproteins. The behaviour of this enzyme in response to the presence or the absence of serum differed somewhat from the other enzymes shown in Table 1. There was a much larger difference apparent between tissue maintained with serum compared with tissue maintained without serum (approx. 4-fold), and the changes observed following switching were not as marked. The reasons for this difference are not clear, but probably reflect differences in the rate of turnover of this enzyme compared with the other glycosyltransferases assayed.

DISCUSSION

Using benzyl β-D-xyloside as an exogenous initiator of chondroitin sulphate synthesis, we have previously demonstrated that bovine articular cartilage maintained in culture with added serum possessed an increased capacity for chondroitin sulphate synthesis compared with tissue maintained in medium alone (Hascall *et al.*, 1983a). The possibility that this observation arose through an increase in the activity of the glycosyltransferase enzymes has been confirmed in the present study. We have demonstrated that three enzymes, namely galactosyltransferase I, galactosyltransferase II and glucuronosyltransferase II, are all increased at least 2-fold in tissue maintained with serum. This suggests that serum induces a co-ordinated increase in both the cellular concentrations of the chondroitin sulphate glycosyltransferases as well as the amount of core protein being processed within the chondrocyte. It remains possible, however, that the measured increase in concentrations of extractable enzymes may simply reflect an overall stimulation of total protein synthesis, the end result being an apparent co-ordination of enzyme activity with synthesis of core protein.

Experiments in which cycloheximide was used to inhibit proteoglycan synthesis demonstrated the presence of a pool of proteoglycan core protein precursor available for processing, subsequent to the inhibition of protein synthesis. This is consistent with previous studies with chondrocytes from the Swarm rat chondrosarcoma (Kimura *et al.*, 1981; Mitchell & Hardingham, 1981), in which the proteoglycan core precursor was shown to have an intracellular half-life of 60–90 min. In explants of bovine articular cartilage, the half-life of the proteoglycan core protein is about 30 min (McQuillan *et al.*, 1984). The calculated half-life of the core protein was shown to be independent of the presence or the absence of serum in the culture medium for up to 6 days in culture, and was also independent of the rate of proteoglycan synthesis. This suggests that the presence of serum, or serum growth factors, stimulates an increase in the amount of proteoglycan core protein processed per unit time, without any apparent change in the kinetics of turnover of the available pool of core protein, and further suggests that the stimulation of proteoglycan synthesis by serum must be manifest at a step before synthesis of core protein.

Alternative explanations for a stimulated rate of proteoglycan synthesis by tissue cultured in the presence of serum are difficult to reconcile with these results. An increased rate of intracellular transport of nascent core

protein or of intermediates in the pathway of proteoglycan synthesis could, in the short term, be a factor in the increased flux of [35 S]sulphate into proteoglycan, but in a steady-state situation, such as in cartilage maintained in culture for several days, an overall increase in synthesis of core protein must exist. Furthermore, an increased rate of transport or utilization of core protein without a concomitant increase in pool size would inevitably lead to a change in the exponential decay constant of the core-protein pool. No such change could be detected.

Another possibility is that culture in the presence of serum in some way altered the transport or metabolism of inorganic sulphate in the chondrocyte so that the specific radioactivity of intracellular phosphoadenosine phosphosulphate was increased. Thus an increased rate of 35 S incorporation would not necessarily reflect an increase in proteoglycan synthesis. This explanation is also unlikely, since incorporation of [3 H]acetate into proteoglycan was also stimulated by serum (McQuillan *et al.*, 1984). Nor can an increase in the pool size of nucleotide sugars readily account for the stimulation of 35 S incorporation into proteoglycan. Any change in nucleotide sugar pool size sufficient to change the rate of 35 S incorporation to the extent observed in this study would be manifest either in an altered glycosaminoglycan chain size or in the degree of substitution of the proteoglycan core with glycosaminoglycan chains. No evidence for such changes has been detected in this culture system (Hascall *et al.*, 1983a).

The slow decay in the rate of proteoglycan synthesis upon exposure of the cultures to actinomycin D (McQuillan *et al.*, 1986) is believed to be a measure of depletion of a pool of mRNA coding for core protein coupled with the subsequent depletion of the pool of core protein itself. It has been previously shown by us (McQuillan *et al.*, 1986) that actinomycin D had no marked effect on the concentrations of chondroitin sulphate glycosyltransferases over the periods of time the experiments described in the present study were performed. In contrast with the pool of proteoglycan core protein precursor, one molecule of mRNA has the potential to code for a large number of core protein molecules. If the number of times mRNA coding for the core protein can be translated is constant, then, under steady-state conditions, the calculated half-life of the pool of proteoglycan mRNA is a measure of the amount of mRNA available at any given time. In the experiments described above, the half-life of core-protein mRNA in tissue maintained in the presence of serum was 8.5 h, compared with 3.8 h for tissue maintained in medium alone, suggesting that serum had stimulated an increase in the amount of mRNA coding for the proteoglycan core protein. This suggests in turn that serum or serum growth factors are active, directly or indirectly, at the level of DNA-dependent RNA synthesis, so as to modulate the amount of core-protein-specific mRNA. On the other hand, an alternative explanation is that the net amount of mRNA coding for the core protein

remained constant in the presence and in the absence of serum, and the different half-lives reflect a stabilization of the pool of these re-usable molecules. It is possible that serum or serum growth factors induce the production of some unspecified factor, the function of which is to enhance the cellular life of fully functional mRNA molecules.

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