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Effect of Glucocorticoids on Glycosaminoglycan Metabolism in Cultured Human Skin Fibroblasts

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Human skin fibroblasts were exposed to 3 anti-inflammatory steroids in order to study their effects on the glycosaminoglycan metabolism. The potent glucocorticoids, fluocinolone acetonide and budesonide, even at low concentrations strongly reduced the accumulation of hyaluronic acid and sulfated glycosaminoglycans in the medium, at the cell surface, and in the cells. Hydrocortisone had considerably less effect. The 3 compartments were not influenced to the same extent and the least inhibition was noted in the cell surface pool. Dermatan sulfate was decreased to the same relative extent in all 3 compartments, while hyaluronic acid and heparan sulfate were specifically retained at the cell surface, explaining why this compartment was less affected than the others.

Dermatan sulfate was studied in more detail regarding effects on its copolymeric structure. Glucocorticoid treatment changed the uronosyl composition of the polysaccharides so that a relative decrease of glucuronic acid residues and a relative increase of iduronic acid residues were noted. This change was most evident in dermatan sulfate of the medium and of the cell surface.

Thus, glucocorticoid treatment not only reduces the quantity of various glycosaminoglycans but also changes the distribution, the relative proportion, and the structure of connective tissue proteoglycans. These effects probably contribute to the development of skin atrophy, which often is observed after long-term treatment with potent glucocorticoids.

The long-term use of potent glucocorticoids often induces profound changes of connective tissue. In skin, such therapy may provoke thinning or, in more severe cases, atrophy of the skin, induction of striae, or impaired wound healing [1]. The mechanisms behind the development of these events are only partly understood. The effects of glucocorticoids on cultured dermal fibroblasts as well as on epidermal cells have been thoroughly investigated. Thus it is already known that glucocorticoids in therapeutic doses can inhibit cell proliferation and mitosis [2], decrease collagen and glycosaminoglycan synthesis [3–7], and in higher doses they also have an inhibitory effect on the general protein synthesis [8]. These effects on cellular processes have been regarded as an explanation of the skin atrophy observed after prolonged treatment with glucocorticoids in vivo.

Model experiments with topical application of potent glucocorticoids to rat skin have shown that loss of glycosaminoglycans and water are among the first changes to appear in skin (Särnstrand, unpublished). It was therefore considered of interest to study the effect of glucocorticoids on the metabolism of glycosaminoglycans in cultured dermal fibroblast. The aim was to see whether glucocorticoid-induced inhibition of glycosaminoglycan metabolism is specific or only related to the general catabolic effects. Three glucocorticoids were tested: hydrocortisone as an endogenous glucocorticoid of low potency, and fluocinolone acetonide and budesonide [9,10] of high anti-inflammatory potency. All these are used in topical dermatologic therapy. The results show that at least the 2 potent glucocorticoids have specific actions on the glycosaminoglycan synthesis and distribution.

MATERIALS AND METHODS

Materials

Papain (E.C. 3.4.4.10, twice crystallized), trypsin (E.C. 3.4.21.4) and L-cystine HCl were purchased from Sigma Chemical Co., St. Louis, Missouri. Eagle's minimal essential medium, calf serum, and all other items needed for cell cultures were obtained from Flow Laboratories, Irvine, Ayrshire, U.K. Carrier-free $Na_2{}^{35}SO_4$ (124 mCi/mmol), D-[1-^{3}H]-glucosamine HCl (2.8 Ci/mmol) and L-[4.5-^{3}H]-leucine (150 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Buchs, U.K. Chondroitinase-AC and -ABC (E.C. 4.2.2.5 and E.C. 4.2.2.4) were bought from Miles Laboratories, Elkhart, Indiana. Sephadex G-50 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden, and DEAE-cellulose DE-52 from Whatman Inc. (WR Balston, Ltd., Maidstone, Kent, UK). Instagel was bought from Packard Instrument AB, Bandhagen, Sweden. Hyaluronate was bought from Sigma, dermatan sulfate and heparan sulfate were prepared from pig skin [11] and from heparin side fraction from bovine lung [12], respectively. Hydrocortisone was obtained from Apoteksbolaget, Sweden, fluocinolone acetonide from Lark, Italy, and budesonide from AB Draco, Sweden [9]. The steroids were prepared as stock solutions in concentration from 5×10^{-2} M to 5×10^{-8} M in absolute ethanol. Other chemicals were of analytical grade.

Culture Methods

Fetal human skin fibroblasts were maintained in 75-cm^2 culture glass flasks as monolayer, as described by Malmström et al [13]. For the different experiments cells were cultured in 2.3-cm^2 (FB-16-24-TC, Linbro) or 25-cm^2 plastic flasks (Falcon). At confluency, sulfate-poor medium was added [12] and after 24 hr exchanged for fresh medium containing steroids at concentrations of 10^{-5}–10^{-11} M. (Final ethanol concentration of 0.02%.) After 24 hr $Na_2{}^{35}SO_4$ (10 μCi/ml) and [^{3}H]-glucosamine (2.5 μCi/ml) were added.

After 48 hr of isotope incorporation the medium was collected and the cell layer was washed twice with 2 ml cold, phosphate-buffered saline (PBS), 137 mM NaCl/3 mM KCl/8 mM Na_2HPO_4/1 mM KH_2PO_4, pH 7.4. The washings were combined with the medium. This fraction was designated the medium. The cells were brought into suspension by treatment with 3 ml prewarmed 0.4% trypsin in PBS for 15 min at 22°C with occasional gentle shaking and then recovered by centrifugation at $600 \times g$ (2000 rpm) for 5 min and washed 4 times with PBS. The washings were combined with the cell-free trypsin digest supernatant. This fraction was designated the cell surface pool. Both the trypsin digest and the medium were centrifuged at $10{,}000 \times g$ for 10 min to remove all cellular debris.

The cells were suspended in 1 ml water and were treated with 5 freeze–thaw cycles or with sonication. This fraction was designated the cell pool.

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Isolation of Glycosaminoglycan

Carrier solution, to give a final concentration of 0.1 mg hyaluronic acid, 0.05 mg dermatan sulfate, and 0.05 mg heparan sulfate, was added to all 3 fractions referred to before papain digestion. Papain (3 × 6 U) and digest buffer were added to give a final concentration of 1.0 M NaCl, 0.05 M EDTA, 0.01 M cysteine-HCl, 0.05 M sodium phosphate, pH 7.0. The samples were then kept at 65°C for 24 hr.

The digest was diluted 10 times and applied to ion-exchange chromatography columns of DE-52 DEAE cellulose (1 × 2.5 cm bed volume) equilibrated with 0.2 M LiCl containing 6 M urea and 0.05 M sodium acetate, pH 4.0. The columns were eluted stepwise with 2 × 10 ml of the equilibrating buffer to get rid of nonincorporated material, followed by 2 × 5 ml 0.35 M 2 × 5 ml 0.4 M; 2 × 5 ml 0.8 M LiCl, all in 6 M urea and 0.05 M sodium acetate, pH 4.0. Hyaluronic acid was obtained mainly in the 0.35 M LiCl fraction while dermatan-chondroitin sulfate and heparan sulfate were obtained in the 0.8 M LiCl fraction.

Estimation of General Protein Synthesis

Total protein synthesis was studied by incubating cultured fibroblasts with [^{3}H]-leucine after preincubation, with the different steroids. After 6 hr of [^{3}H]-leucine incubation the cells were harvested as above. The cells were suspended in 10% trichloroacetic acid (TCA). After sonication for 20 sec the samples were centrifuged at 8,000 × g for 5 min. The pellet was washed 3 times with 10% TCA and finally dissolved in 0.5 ml of 0.5 M sodium hydroxide.

Analytical and Separatory Methods

Ion-exchange chromatography was carried out on columns (10 × 25 mm) of DE-52 DEAE cellulose as described above. Before structural studies the sulfated glycosaminoglycans were further purified on columns (10 × 25 mm) of DE-52 cellulose ion-exchange as described by Sjöberg et al [14]. Heparan sulfate and dermatan sulfate were obtained in the 3.0 M pyridine acetic acid fraction.

Gel chromatography was carried out on columns (8 × 144 mm) of Sephadex G-50 eluted by 0.05 M NH_4HCO_3 in a rate of 10 ml/hr.

Radioactivity was determined in a Packard (2650) Tri-Carb liquid-scintillation counter with automatic quench-correction.

Protein was determined as described by Lowry et al [15] with bovine serum albumin as standard, and the DNA content by the method of Hill and Whatley [16].

Structural Studies

One portion of the sulfated glycosaminoglycans was treated with nitrous acid which depolymerizes heparan sulfate but does not attack galactosaminoglycans [17]. Another portion was digested with chondroitinase-ABC as described by Carlstedt et al [18]. The products of these degradations were subjected to gel chromatography on Sephadex G-50. In the former procedure dermatan sulfate is obtained in the void volume and heparan sulfate split products are retarded. The latter procedure yields intact heparan sulfate in the void volume whereas degraded dermatan sulfate was more retarded. Dermatan sulfate was also subjected to further digestion with chondroitinase-AC [18]. This treatment was followed by gel chromatography on Sephadex G-50 in order to study the distribution of glucuronic acid and iduronic acid containing saccharides.

Statistical Method

All values reported represent the mean ± the standard error of the mean (SEM) of at least 10 observations. Significances are calculated according to two-sided paired *t*-tests. Differences were considered significant when $p < 0.05$.

RESULTS

Effect of Glucocorticoids on the Accumulations of Labeled Glycosaminoglycans in the Cell Culture

Budesonide, a synthetic potent glucocorticoid [9], induced a dose-response related decrease of the accumulation of ^{3}H-labeled glycosaminoglycans in the medium, in the cell surface fraction (trypsin digest of the cell layer), and in the cells (cells obtained after trypsin digestion) (Fig 1). The decrease was most prominent in the medium; a 10^{-5} M concentration of budesonide induced a 70% inhibition of both ^{3}H-labeled hyaluronic acid and sulfated ^{3}H-labeled glycosaminoglycans. The dose-related decrease of ^{3}H accumulation was similar for hyaluronic acid and sulfated ^{3}H-labeled glycosaminoglycans. A dose-response related inhibition was also obtained with [^{35}S]-sulfate incorporation (Fig 1*a*) into the glycosaminoglycans of the medium (Table I), but the ^{35}S-incorporation was less inhibited than the corresponding ^{3}H-incorporation (Table I, Fig 1). The accumulation of the cell surface-associated glycans was not inhibited to the same extent as that of the glycans of the medium (Fig 1*b*) and 10^{-5} M of budesonide caused only a 25–60% decrease (Table I). The accumulation of glycans in the cells was inhibited to an

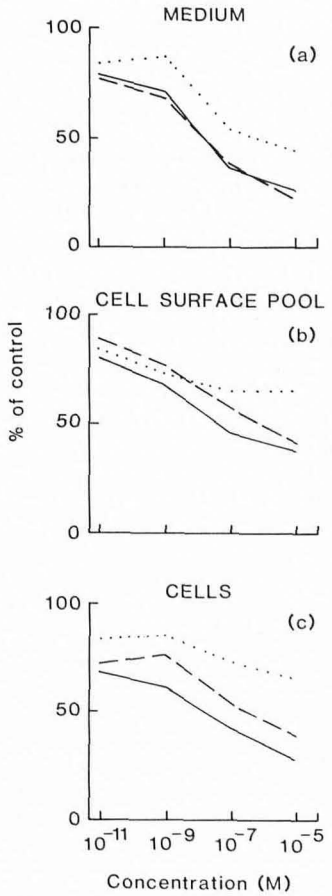

FIG 1. Effects of budesonide on glycosaminoglycan accumulation in the medium (*a*), the cell surface fraction (*b*), and the remaining cells (*c*). Glycosaminoglycan accumulation during a 48-hr pulse of $^{35}SO_4$ and [^{3}H]-glucosamine was measured after 24 hr of budesonide preincubation. The glycans were separated into nonsulfated and sulfated fractions as described in Materials and Methods. The values were obtained from one typical experiment and are given as percentage of control. ^{3}H-labeled hyaluronic acid, ————; ^{3}H-labeled sulfated glycosaminoglycans, – – –; and ^{35}S-labeled glycosaminoglycans, · · · · ·

TABLE I. *Effect of glucocorticoid treatment on the amount of radioactive glycosaminoglycan in the cell culture*

Fraction	^{3}H-labeled hyaluronic acid (%)	^{3}H-labeled sulfated glycosamino-glycans (%)	$^{35}SO_4$-labeled glycosamino-glycans (%)
Whole culture	34 ± 2	34 ± 2	65 ± 3
Medium	31 ± 2	30 ± 2	60 ± 5
Cell surface pool	62 ± 10	42 ± 3	76 ± 6
Cells	39 ± 6	39 ± 6	79 ± 8

Fibroblasts in culture were incubated with budesonide (10^{-5} M), $^{35}SO_4$- and ^{3}H-labeled glucosamine as described in Materials and Methods. Hyaluronic acid and sulfated glycosaminoglycans were isolated by ion exchange chromatography. Mean ± S.E.M. from 6 experiments are given in percent of untreated cultures.

intermediate extent, compared with the medium and the cell surface fractions (Fig 1*c*).

In order to investigate whether different glucocorticoids influence cultured fibroblasts differently, 3 steroids were tested with respect to the accumulation of glycosaminoglycans in the medium. As can be seen in Fig 2*a*, hydrocortisone affected only the incorporation of [^{3}H]-glucosamine into hyaluronic acid to a small extent at the concentration 10^{-5} M. Budesonide and fluocinolone acetonide, however, strongly decreased the glycosaminoglycan secretion, and at 10^{-5} M they inhibited hyaluronic acid synthesis by at least 60%. At lower concentration smaller effects were noted especially for budesonide (Fig 2*a*). When the accumulation of ^{3}H-labeled sulfated glycosaminoglycans was examined, very similar dose-response curves for the respective steroids to those of hyaluronic acid synthesis were obtained (Fig. 2*b*). Again fluocinolone acetonide strongly inhibited secretion of ^{3}H-labeled glycosaminoglycans into the medium; thus, no change of the relative amounts of hyaluronic acid and sulfated glycans could be observed in the medium with respect to the glycans. The incorporation of $^{35}SO_4$ into excreted glycosaminoglycans showed a similar inhibition, although to a smaller extent, than the [^{3}H]-glucosamine incorporation. Hydrocortisone was nearly ineffective at all concentrations, while fluocinolone acetonide and budesonide inhibited the synthesis 40–50% at 10^{-5} M (Fig 2*c*).

Both protein- and DNA-content of treated and control cultures were measured. No change in total amount of either protein or DNA between control and budesonide-treated cultures was noted (Table II). Furthermore, no effect caused by budesonide on the general protein synthesis, measured by [^{3}H]-leucine incorporation into TCA-precipitable protein, was observed (Table II).

Effect of Glucocorticoid Treatment on the Distribution of Various Glycosaminoglycans in the Cell Culture

After 48 hr of incorporation of radioactive precursor the main part of the synthesized hyaluronic acid and sulfated glycosaminoglycans was found in the medium (Table III). Budesonide treatment (10^{-5} M) changed the distribution of glycans between the various compartments to some extent. After treatment a relative decrease of the glycans of the medium and a corresponding increase of those of the cell surface fraction were noted (Table III). The proportion of glycosaminoglycans intracellularly was not changed to any large extent (Table III). Both hyaluronic acid and sulfated glycosaminoglycans of the medium were decreased to the same degree by the treatment. In the cell surface fraction, however, hyaluronic acid was nearly doubled (Table III). In absolute amounts only a 30–40% decrease of hyaluronic acid in the cell surface fraction was noted, while it was decreased 60–80% in the medium and cell fraction by treatment.

TABLE II. *Effect of glucocorticoid treatment on DNA and protein content in cells*

	Budesonide concentration			
	10^{-11} M	10^{-9} M	10^{-7} M	10^{-5} M
DNA n = 6	93 ± 7	98 ± 3	94 ± 6	94 ± 6
Protein Folin value n = 4	110 ± 19	95 ± 10	107 ± 15	96 ± 12
Protein [^{3}H]-leucine incorporation n = 4	N.D.	95 ± 8	102 ± 4	112 ± 10

Cultures were preincubated with budesonide for 24 hr. DNA and protein were determined after additional 48 hr incubation. [^{3}H]-leucine incorporation into cellular proteins was measured after preincubation with a 6-hr pulse. Similar effects on [^{3}H]-leucine incorporation were found in the medium and cell surface fraction. The results are given in mean ± S.E.M. as percent of untreated cultures. N.D. = not done.

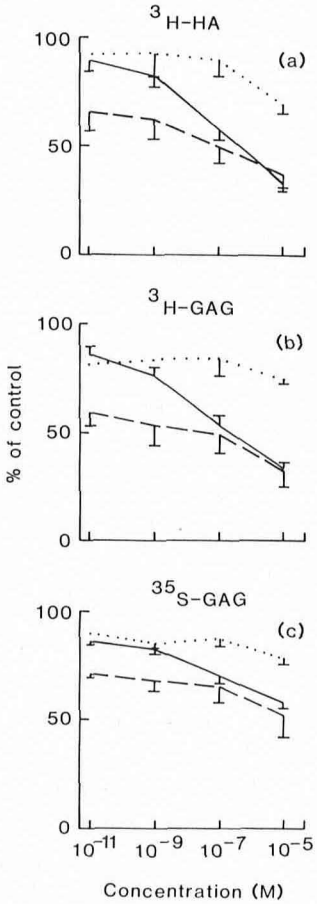

FIG 2. Screening of glucocorticoids effect on hyaluronic acid (*a*), ^{3}H-labeled sulfated glycosaminoglycans (*b*), and $^{35}SO_4$-labeled sulfated glycosaminoglycans (*c*) in the medium. Fibroblasts in culture were preincubated with steroids. After 24 hr $^{35}SO_4$- and ^{3}H-labeled glucosamine were added, and after an additional 48 hr sulfated glycosaminoglycans and hyaluronic acid were isolated as described in Materials and Methods. Effect of hydrocortisone, · · · · · ·; effect of budesonide, ————; effect of fluocinolone acetonide – – –.

TABLE III. *Distribution of [^{3}H]-glycosaminoglycans in untreated and glucocorticoid-treated cultures*

Fraction	Total [^{3}H]-glycosaminoglycans		[^{3}H]-hyaluronic acid		[^{3}H]-sulfated glycosaminoglycan	
	Untreated (%)	Treated (%)	Untreated (%)	Treated (%)	Untreated (%)	Treated (%)
Medium	72 ± 1	67 ± 2	80 ± 2	74 ± 3	55 ± 3	50 ± 3
Cell surface pool	15 ± 2	20 ± 3	9 ± 1	15 ± 3*	27 ± 4	31 ± 4*
Cells	13 ± 2	13 ± 2	12 ± 2	11 ± 1	18 ± 3	20 ± 4

Cultures were treated with budesonide 10^{-5} M and after 24 hr [^{3}H]-glucosamine was added. After 48 hr hyaluronic acid and sulfated glycosaminoglycans were isolated as described in Materials and Methods. The data are compiled from 10 different observations and given as percentage of the total amount of glycosaminoglycan, hyaluronic acid, and sulfated glycosaminoglycan, respectively.

* $p < 0.05$.

Glucocorticoid-Induced Changes of the Relative Proportion and Distribution of Dermatan Sulfate and Heparan Sulfate

The effect of budesonide treatment (10^{-5} M) on the composition of the sulfated glycosaminoglycans was assessed by specific degradations. The relative amounts of heparan sulfate and dermatan sulfate were determined either by nitrous acid degradation or by chondroitinase-ABC digestion followed by chromatography on Sephadex G-50, which separates degraded from nondegraded material. Budesonide treatment affected the 2 polysaccharides differently (Table IV). A relative decrease of heparan sulfate compared to dermatan sulfate in the medium was noted, while the proportion of heparan sulfate in the cell surface fraction increased from 42% to 62%. The most marked change in the proportion of heparan sulfate was noted intracellularly where a 2-fold relative increase was recorded.

In total amounts, the accumulation of dermatan sulfate decreased to the same extent, 60–70% compared to control cultures in all fractions, while the accumulation of heparan sulfate decreased 70–80% in the medium, 30–40% in the cell surface-derived fraction, and only 20–30% intracellularly.

TABLE IV. *Effect of glucocorticoids on the relative amounts of heparan sulfate in the various compartments*

Fraction	Untreated (%)	Treated (%)
Medium	18	12
Cell surface pool	42	62
Cells	17	32

Fibroblasts in culture were treated with budesonide (10^{-5} M) and [^{3}H]-glucosamine as described in Materials and Methods. Sulfated glycosaminoglycans were isolated from the 3 compartments as described. The proportion of heparan sulfate of the sulfated glycosaminoglycans was determined by either nitrous acid degradation or chondroitinase-ABC digestion followed by chromatography on Sephadex G-50. The two methods gave identical results. Data from one typical experiment are given as percentage of total sulfated glycosaminoglycans.

Effect of Glucocorticoid Treatment on the Copolymeric Structure of Dermatan Sulfate

Dermatan sulfate obtained after nitrous acid degradation was subjected to digestion by chondroitinase-AC. This enzyme cleaves glycosidic linkages between N-acetyl-galactosamine and glucuronosyl residues while those to iduronosyl residues are left intact. Thus, after digestion and separation on a column of Sephadex G-50, saccharides chromatographing in the total volume arise from glucuronosyl-containing sequences, while those in the void volume are composed mainly of L-iduronosyl residues. In nonsteroid-treated cultures the glycans of the cells contained mainly iduronic acid residues (Fig 3*c*), while glycans of the medium contained a somewhat higher proportion of D-glucuronosyl residues (Fig 3*a*). The cell surface-associated gly-

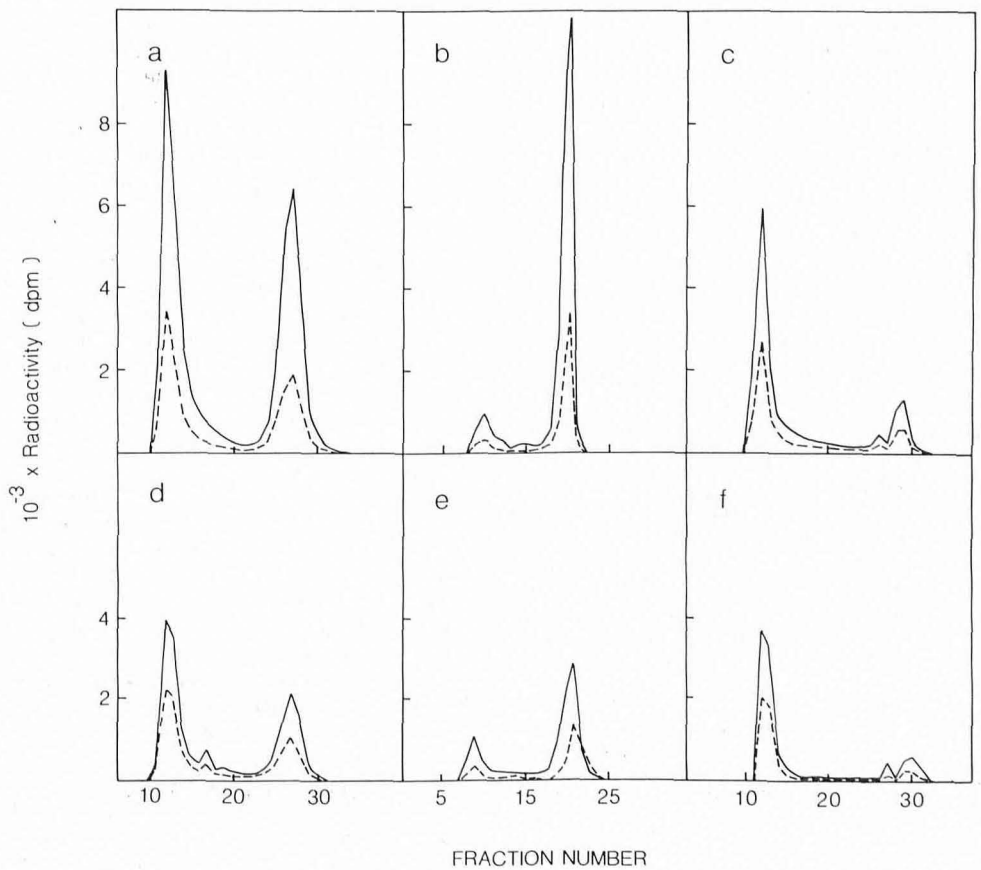

FIG 3. Gel chromatograms of chondroitinase-AC digests of dermatan sulfate obtained from the medium (*a* and *d*), the cell surface pool (*b* and *e*), and cells (*c* and *f*) of control and glucocorticoid (budesonide 10^{-5} M)-treated fibroblast cultures, respectively. Dermatan sulfate was isolated from various compartments of treated (*d–f*) and nontreated (*a–c*) fibroblast cultures. The copolymeric structure was elucidated by chondroitinase-AC digestion followed by chromatography on a column (0.8 × 140 cm) of Sephadex G-50. The flow was 10 ml per hr, and fractions of 2.6 ml (*a*, *d*), 3.4 ml (*b*, *e*), and 2.4 ml (*c–f*) were collected. The material obtained in the void volume of the column is regarded as iduronic acid-containing components, while the included material is regarded as glucuronic acid-containing. This approximation introduces a small error as the void volume can contain maximally 12% of glucuronic acid. The included material is more than 90% disaccharides which originate from glucuronic acid residues. The longer oligosaccharides (less than 10%) contain small amounts of iduronic acid residues [18]. Radioactivity of [^{3}H]-glucosamine, ————; radioactivity of [^{35}S]-sulfate, - - - - -.

TABLE V. *Effect of glucocorticoid treatment on the copolymeric structure of dermatan sulfate*

Fraction	Iduronic acid-containing components (%)	Glucuronic acid-containing components (%)
Medium	45	33
Cell surface pool	108	48
Cells	69	57

Fibroblasts in culture were treated with budesonide (10^{-5} M), $^{35}SO_4$ and [^{3}H]-glucosamine, and sulfated glycosaminoglycans were isolated. Heparan sulfate was removed after nitrous acid treatment by chromatography on a column of Sephadex G-50. The copolymeric structure of dermatan sulfate was investigated after chondroitinase-AC digestion followed by gel chromatography (Fig 3). The material in the V_0-volume is regarded as iduronic acid components, while those chromatographing as retarded are calculated as glucuronic acid-containing material. The data from one typical experiment are given as percentage of the material obtained in the V_0 and retarded, respectively, of untreated cultures.

cans were rich in D-glucuronosyl residues with a large proportion of the radioactivity chromatographing as disaccharides after chondroitinase-AC digestion (Fig 3*b*).

Budesonide treatment (10^{-5} M) changed the copolymeric structure of dermatan sulfate in the direction of a decreased proportion of D-glucuronosyl residues (Fig 3*d–f*, Table V). Thus, the amount of D-glucuronic acid-containing disaccharides of glycans in the medium decreased more (down to 33% of the control) than did the iduronic acid (down to 45% of the control). Such a change was evident also for glycans of the cells. The dermatan sulfate of the cell surface is the fraction that normally contains most glucuronic acid residues (Fig 3*b*). In this fraction budesonide induced the largest change in relative composition (Fig 3*e*, Table V). Compared to control cultures, no decrease of the iduronosyl material was observed, while a 48% decrease of the glucuronosyl-containing components was recorded. Thus, the glucocorticoid treatment resulted not only in an absolute decrease but also in a change of the copolymeric structure of dermatan sulfate, especially in the cell surface fraction.

DISCUSSION

Fibrous connective tissue is difficult to study metabolically in vivo and therefore many workers have turned their attention to cultured fibroblasts [6] for investigating the effects of glucocorticoids. Depending on the actual glucocorticoid, its concentration, and preincubation time, varying effects on the glycosaminoglycan synthesis have been reported, from slightly stimulatory to highly inhibitory [19]. In the present study a clear inhibition of the glycosaminoglycan-synthesis and -excretion into the medium was demonstrated at concentrations $\geq 10^{-5}$ M for hydrocortisone, $\geq 10^{-9}$–10^{-7} M for budesonide, and even down to 10^{-11} M for fluocinolone acetonide. There was a deviating slope of the dose-response relationship for budesonide compared with those of the other 2 glucocorticoids. This deviation seems not to depend on varying biotransformation of the glucocorticoids during the incubation period, as neither hydrocortisone nor budesonide [20] (Särnstrand, unpublished) nor fluocinolone acetonide [21] are biotransformed to any greater extent by fibroblasts.

It should be noted that the effect of the 2 potent glucocorticoids on glycosaminoglycan synthesis and secretion was obtained at concentrations where no effect of general protein synthesis could be recorded. Furthermore, no decrease of either DNA nor of protein content was detected. This is in accordance with other investigations [4]. The inhibition of glycosaminoglycan synthesis therefore seems to be a result of an effect on some specific step in the synthesis or on the formation of precursors.

In the present study the fibroblasts were preincubated for 24 hr with glucocorticoids to ensure a sufficient lag time for the glucocorticoid action before the radioactive precursors were added. The results demonstrate that the incorporation of [^{3}H]-glucosamine into hyaluronic acid and sulfated glycosaminoglycans of the medium was inhibited to the same extent. This suggests that a common and early step in their synthesis was affected. However, the $^{35}SO_4$ and [^{3}H]-glucosamine incorporation into the sulfated glucosaminoglycans differed. This can be explained either by an oversulfation or by a change in the specific activity of the nucleotide precursors. Preliminary data indicated that the specific activity of UDP-N-acetylhexosamine pool was diminished by steroid treatment (Särnstrand, unpublished). Although there was a difference in the decrease of ^{3}H and $^{35}SO_4$ incorporation, the relative composition and distribution of $^{35}SO_4$-labeled glycans were similar to those of ^{3}H-labeled glycans. As a majority (65–80%) of the glycans are secreted to the medium, it was considered most relevant to use a screening method to determine the effect of various substances on glycosaminoglycan metabolism. The adopted method, involving preincubation with the test substance, precursor incorporation, and isolation of labeled glycosaminoglycans, offers the possibility to screen effects on cells from different tissues and animals. The method might be of special interest for the development of glucocorticoid derivatives with relatively less effect on connective tissue.

The effects of one of the glucocorticoids (budesonide) were studied in more detail. It became evident that the various polysaccharides on the cell surface and in the cell fraction were influenced differently compared with those of the medium fraction. Especially noticeable was the smaller extent of inhibition of glycosaminoglycans in the cell surface fraction, which was due to smaller effect on hyaluronic acid and heparan sulfate than on dermatan sulfate. Heparan sulfate is most likely an integral macromolecule of the cell membrane [22] and it has been ascribed hypothetical functions in cell-to-cell interaction and shielding of the cell surface. Hypothetical receptors at the cell surface have also been described for hyaluronic acid [23]. In any event, these glycans might have important functions at the cell surface and are therefore selectively retained during glucocortocoid treatment. Dermatan sulfate, which probably has its main function in the matrix, in vivo might not be as important for the immediate survival of the cell.

The function of dermatan sulfate proteoglycans in fibrous connective tissue is obscure. They have been ascribed functions in waterbinding, self-self interaction to form gels, and, probably most important, an effect on collagen fibril formation [24–26]. In some of these functions the copolymeric structure of the side chains and mixture of different proteoglycans is of importance. A changed structure might then impair or change the functions of the whole tissue. The effect of glucocorticoid treatment on the copolymeric structure of dermatan sulfate was therefore investigated. A decrease of glucuronic acid-containing disaccharides compared to iduronic acid-containing ones was then detected. This effect was especially conspicuous in the glycans of the cell surface. At the present stage it is difficult to judge whether the change affects all dermatan sulfates or only a fraction of them and what effect this change has on cell function. Recently, it has been shown that skin fibroblasts in culture synthesize 2 types of proteoglycans, one containing side chains rich in glucuronic acid and one that is rich in iduronic acid [18]. Preliminary data indicate that the former is more inhibited by budesonide than the latter (Särnstrand, unpublished).

In conclusion, glucocorticoid treatment not only changes the amount of proteoglycans in and around the treated cells but can also induce a change in distribution between matrix and cell-pericellular domain and a change of the dermatan sulfate structure. The decreased content of proteoglycans combined with a changed proportion and change of structure of various species are most likely important factors for the development of skin atrophy.

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Identification, Characterization, and Partial Purification of Mammalian Skin Wound Hyaluronidase

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A recently described mammalian wound hyaluronidase is successfully characterized and partially purified in the current study. Peak enzyme activity occurred on postwound day 7, pH optimum 4.5. Both crude and purified wound enzyme exhibited endoglycosidic activity against hyaluronate and chondroitin-4-sulfate but not against chondroitin-6-sulfate or dermatan sulfate. A 5.3-fold increase in activity was obtained by the DEAE-Sephadex purification technique described. Polyacrylamide gel electrophoresis yielded a single major band near the gel's midrange and one minor band of lesser electrophoretic mobility. These enzyme characteristics support a biochemical analogy between tissue repair in skin and numerous developmental systems and may also provide a simple means for enzymatic differentiation among chondroitin sulfate isomers.

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Abbreviations:

GAG: glycosaminoglycan

PAGE: polyacrylamide gel electrophoresis

In mammalian wound repair, early cellular proliferation and ground substance synthesis are followed by the resorptive and remodelling processes which collectively constitute wound "maturation." Mechanisms which control the collagenous, cellular, and glycosaminoglycan (GAG) composition of wound granulation tissue remain unknown.

Balazs and Holmgren [1] suggested that wound granulation tissue contained predominantly nonsulfated GAG during the