The Effect of Platelet-Derived Growth Factor on Cell Division and Glycosaminoglycan Synthesis by Human Skin and Scar Fibroblasts*

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The effect of platelet-derived growth factor (PDGF) on cell division and glycosaminoglycan (GAG) synthesis by fibroblasts isolated from skin and scar was measured. We found that PDGF stimulates cell division more efficiently in normal skin fibroblasts than in scar fibroblasts and decreases GAG synthesis in skin and scar fibroblasts. Using a 4-h pulse label with [^{3}H]thymidine ([^{3}H]Thd) following a 20-h incubation of confluent monolayer cultures with 0–5 units PDGF/ml Dulbecco's modified Eagle's medium, we found a concentration-dependent increase in [^{3}H]Thd incorporation. After incubation of fibroblasts with [^{3}H]glucosamine and $^{35}SO_4$ in the presence or absence of PDGF, labeled constituents were isolated from the extracellular, pericellular, and cellular fractions by pronase digestion and column chromatography on Sepharose CL4B or DEAE-cellulose and analyzed by cellulose acetate electrophoresis. The presence of PDGF decreased the total amount of ^{35}S incorporated into macromolecules by skin and scar fibroblasts and resulted in an altered distribution of labeled GAGs. Dermal fibroblasts exposed to PDGF for 24 h incorporated a greater percentage of radiolabeled ^{35}S into dermatan sulfate prime (DS') and less into dermatan sulfate (DS) in the extracellular fractions and a greater percentage of ^{35}S into heparan sulfate (HS) in the pericellular fractions than did parallel cultures grown in the absence of PDGF. It is thought than PDGF may have an effect on scar formation by increasing the fibroblast population in the wound tissue and by affecting the total amount and types of matrix components synthesized. *J Invest Dermatol 89: 93–99, 1987*

The events following injury, including inflammation and repair, serve to promote wound healing. These events involve a number of factors that modulate the metabolic activities of dermal fibroblasts responsible for the synthesis of the scar extracellular matrix macromolecular constituents. To explore the role of platelet-derived growth factor (PDGF) on scar formation, we compared cell division and glycosaminoglycan (GAG) synthesis by fibroblasts isolated from skin and scar tissues in the presence and absence of PDGF.

As a result of injury, blood vessels rupture and bleeding occurs. When platelets come in contact with the extravascular subendothelium, they adhere to collagen, undergo a disc to sphere transformation, and send out long thin pseudopods [1]. The collagen-induced adherent platelets release a variety of factors that promote the formation of a hemostatic plug [2], stimulate mitogenesis [3–7] and chemotaxis [8–10] in fibroblasts, increase vascular permeability, and thus contribute to the inflammatory response [11].

The repair process in which dead cells are replaced by viable tissue occurs in two phases [12,13]. The first is a proliferative phase in which a highly vascular connective tissue matrix is established. During this phase, contraction decreases the area of the wound and epithelialization establishes a cover. There then is an extended remodeling phase involving both synthesis of components and reorganization of these constituents to form mature scar. The fibroblasts present in the new scar are the cells responsible for the biosynthesis of the matrix structural components. These cells, therefore, play a key role in controlling the structure of the repair tissue.

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Abbreviations:
C: cellular pool
C6S: chondroitin-6-sulfate
CPC: cetylpyridinium chloride
CS: chondroitin sulfate
DMEM: Dulbecco's modified Eagle's medium
DS: dermatan sulfate
DS': dermatan sulfate prime
GAG: glycosaminoglycan
HA: hyaluronate
HBSS: Hanks' basic salt solution
HP: heparin
HS: heparan sulfate
HSc: hypertrophic scar
M: extracellular pool
P1: fractions containing only [^{3}H]glucosamine
P2: fractions containing both ^3H and ^{35}S
PDGF: platelet-derived growth factor
PG: proteoglycan
T: pericellular fraction

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The extracellular matrix of skin and scar tissues is composed of collagen, glycoproteins, hyaluronate (HA), and proteoglycans (PGs) and the types and amounts of these constituents in hypertrophic and keloid scars differ from that of normal skin. In hypertrophic and keloid scars, the collagen is arranged in whorls or nodules rather than in parallel arrays [14]. Hypertrophic scars (HSc) contain higher levels of chondroitin sulfate (CS) than does normal skin [15–17]. There is some controversy about the concentration of HA; Shetlar et al [17] reported lower levels of HA in HSc whereas Donoff et al [15] reported higher levels than in normal skin. Fibronectin, a major glycoprotein of the extracellular matrix, appears to be more abundant in HSc than in normal skin [18].

The objective of this study was to determine whether PDGF, which is released early in the wound healing process by platelets, affects fibroblasts isolated from skin and scar tissue with respect to cell division and GAG biosynthesis.

MATERIALS AND METHODS

Cell Culture Human fibroblasts used in this study were obtained from explant culture of human skin and scar tissues removed surgically as a part of patient treatment. The tissue used to initiate each cell line is described in Table I. The cultures were initiated in Dulbecco's modified Eagle's medium (DMEM) (Grand Island Biological Co., Grand Island, New York) plus 10% NuSerum (Collaborative Research Inc., Lexington, Massachusetts) supplemented with penicillin and streptomycin (MA Bioproducts, Walkersville, Maryland). The cultures were weaned from the antibiotics as early as the second week of the explant culture and were then maintained without antibiotics. The explant cultures reached confluency at about 4 weeks; they were subsequently split 1:2 or 1:3 every 5–7 days or as needed. All the experiments reported were done with cells in subpassages (2–14).

The Effect of PDGF on Cell Division

Incorporation of [^{3}H]Thd: Skin and scar fibroblasts were plated in 96-well microtiter plates at 1–2 × 10^4 cells/well in DMEM + 1–2% NuSerum. After 2–4 days the cells were fed with 0.1 ml incubation medium, DMEM + 1 or 2% NuSerum + 0, 0.5, 1.0, 2.0, or 4.0 units PDGF (Collaborative Research, Inc.)/ml medium and allowed to incubate for 20 h. After that time thymidine, [methyl-^{3}H]Thd (sp act 20.0 Ci/mM New England Nuclear, Boston, Massachusetts) was added to give a concentration of 5 μCi/ml. After a 4-h pulse, the medium containing unincorporated label was removed and the plates were rinsed repeatedly with cold Hanks' basic salt solution (HBSS) (MA Bioproducts). These conditions have been shown to wash out unincorporated [^{3}H]Thd (unpublished results). The cells were then lysed by suspension in 50 μl 1 N NaOH and the cell lysate and one water rinse of the well were combined and the incorporated [^{3}H]Thd measured in 3.5 ml scintiverse scintillation cocktail with a Beckman LS-250 scintillation counter.

Table I. Effect of Platelet-Derived Growth Factor (PDGF) on [^{3}H]Thymidine Incorporation by Human Fibroblasts

Cell Line	Percent of Control	N
Normal skin		
3285	626	3
603	1073	3
$\bar{x}$	850	
Normal scar		
2356	150	5
1722	248	5
289	279	6
$\bar{x}$	226	
Hypertrophic scar		
2439	133	2
2726	146	2
2599	160	8
SW-1	201	6
$\bar{x}$	160	

Confluent monolayer cultures of fibroblasts were incubated in Dulbecco's modified Eagle's medium + 1% Nu Serum + 1 unit PDGF/ml for 20 h. The cultures were then pulse labeled with [^{3}H]Tdh for 4 h. The cells were rinsed, solubilized, and the cpm ^{3}H determined. The cpm from PDGF-treated cultures were compared to untreated cultures and the results are given as percent of control (cpm PDGF-treated/cpm control) × 100. N = number of determinations. $\bar{x}$ = mean.

Glycosaminoglycans Biosynthesis

Incorporation of ^{35}S Into Macromolecules: Confluent cultures of human scar fibroblasts in 30- and 60-mm plates in DMEM + 1 or 2% NuSerum containing 10 μCi sodium sulfate (^{35}S, sp act 280–350 mCi/mM, New England Nuclear)/ml medium were incubated for 24 and 48 h in the absence or presence of 1 unit PDGF. The cells in each plate were counted in a Coulter counter after the conditioned medium plus 1 rinse with HBSS per plate were pooled. The samples were dialyzed against 0.05 M Na_2SO_4 and then extensively against water. The dialysate was concentrated by freeze drying, and the incorporated ^{35}S was measured in a Beckman LS-250 scintillation counter.

Other conditioned medium plus buffer rinse samples were digested with 100 μg pronase (Calbiochem-Behring Corp., La Jolla, California) at 37°C for 16 h. A second portion of pronase (100 μg) was added and the mixture was incubated for an additional 4 h when the reaction was stopped by boiling for 2 min. Twenty micrograms of chondroitin-6-sulfate (C6S) or 10 μg heparin (HP) (Sigma Chemical Co., St. Louis, Missouri) was added as a carrier to each sample and then cetylpyridinium chloride (CPC; Sigma) was added to achieve a concentration of 0.5% w/v. The CPC-GAG complexes were collected by centrifugation at 350 *g* or 1500 *g* for 30 min. The pellets were rinsed with 1% CPC to insure the removal of unincorporated $^{35}SO_4$. The pellets were dissolved in 100 μl of 1 M or 2 M NaCl, 10 vol of ETOH was added, and the samples were kept at 4°C overnight. The precipitated GAGs were collected by centrifugation at 350 *g* or 1500 *g* for 30 min. The ^{35}S radioactivity in the ETOH precipitate was then determined. In other samples, the HA was separated from the sulfated GAGs by solubilization of the CPC-HA complex in 0.2 M NaCl. The remainder of the GAGs were then isolated as described above. The GAG content of different fractions was determined by cellulose acetate electrophoresis.

Preparation of Radiolabeled GAG Fractions: Confluent monolayer cultures of human fibroblasts were incubated with DMEM containing 5 μCi glucosamine hydrochloride, D-[6-^{3}H(N)]^{3}H-glucosamine (sp act 31.3 Ci/mM, New England Nuclear) and 10 μCi $^{35}SO_4$/ml and 1% NuSerum in the absence or presence of 1 unit PDGF. The labeled GAGs were harvested from the extracellular (M), pericellular (T), and cellular (C) pools as described previously [19]. Briefly, M included the conditioned medium, T, a trypsinate digest of the cells, and C, a NaOH cell lysate. Each M, T, and C fraction was pronase treated, and freeze-dried in preparation for further fractionation.

Gel Filtration Chromatography: Glycosaminoglycan constituents were fractionated on a column packed with Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala, Sweden) as described previously [19]. The fractions containing only [^{3}H]glucosamine were pooled (P1) separately from those containing both ^{3}H and ^{35}S (P2).

Ion Exchange Chromatography: In other experiments, GAG constituents were also fractionated on columns (0.8 × 18 cm) packed with DE52 (Whatman Chemical Separation Ltd.) and eluted in a stepwise manner with 3 column volumes each of (A) 6 M urea, (B) 6 M urea, 0.2 M NaCl, and (C) 6 M urea, 2 M NaCl. Following elution of the column by gravity flow, the constituents eluted with the 3 elutrient solutions were collected in single fractions.

Analysis of GAGs: The GAG content of the various samples was determined by electrophoresis on cellulose acetate plates [20,21].

The identity of each band was verified by comparisons with reference GAG samples as well as selective digestion with chondroitinase ABC (Seikagaku Kogyo Co., Ltd., Tokyo, Japan) and streptomyces hyaluronidase (Calbiochem-Behring Corp.). The quantities of radioactivity in individual GAG bands were determined as described previously [19] by cutting up the cellulose plates, using the mobilities of the reference GAGs as a guide. After placing the cut pieces in scintillation cocktail, the quantity of radioactivity was determined by counting in a Beckman LS-250 apparatus. Background counts were determined by measuring the radioactivity in unlabeled reference GAG bands.

RESULTS

The Effect of PDGF on Cell Division

[^{3}H]Thymidine Incorporation: The increase in [^{3}H]Thd incorporation in the presence of 1 unit PDGF/ml over that of untreated controls is shown in Table I. Normal skin fibroblasts were more sensitive to the mitogenic effect of PDGF than normal scar fibroblasts and normal scar fibroblasts were more responsive than hypertrophic fibroblasts.

The increase in [^{3}H]Thd incorporation in the presence of increasing concentrations of PDGF is shown in Fig 1.

The Effect on GAG Synthesis by PDGF

Distribution of Radiolabel Among the Various Fractions: The distributions of ^{3}H and ^{35}S among the M, T, and C fractions of HSc fibroblasts cultured in the presence and absence of PDGF are listed in Table II. The M fraction contained the highest proportion of both ^{3}H and ^{35}S. There was no significant difference in the distribution of radiolabel among the fractions from PDGF-treated and control cultures.

A decrease was observed, however, in the ^{35}S incorporated into medium-nondialyzable and into CPC- and ETOH-precipitable constituents (Table III). In 3 of the HSc cell lines, the average incorporation was 62% of the control cultures in the absence of PDGF. In a fourth HSc cell line and in cell lines from a normal scar and normal skin, the decreases in incorporation compared with controls were small.

Analysis of the GAGs

Gel Filtration Column Chromatography: After pronase digestion the radiolabeled constituents from each of the M, T, and C fractions from the normal skin cell line 3285 cultured in the presence and absence of 1 unit PDGF/ml DMEM + 1% NuSerum were dialyzed extensively and subjected to column chromatography. The elution profiles are shown in Fig 2. Each sample contained a peak of ^{3}H radioactivity eluted at the Vo and a peak of material containing ^{35}S radioactivity eluted in the partially included volume. The column fractions indicated were pooled to yield the fractions P1 and P2.

The profiles of the ^{35}S-labeled GAGs found in the M, T, and C fractions were similar in comparison between the PDGF-treated and untreated normal skin fibroblast cultures (Fig 2). The peak Kav values for the M and T fractions with and without PDGF were 0.68. The Kav values of the C fractions with and without PDGF were 0.82. A reference C6S used to calibrate the column had a Kav of 0.60.

Ion Exchange Column Chromatography: After pronase digestion, the radiolabeled constituents from each M, T, and C fraction from the HSc cell line 2266 cultured in the presence and absence of 0.5 unit PDGF/ml DMEM + 1% NuSerum was dialyzed extensively and subjected to ion exchange column chromatography. Three fractions (A, B, and C) were obtained from each sample as described under *Materials and Methods*. The first fraction (A) eluted with 6 M urea contained no radioactivity. The second fraction (B) eluted with 6 M urea, 0.2 M NaCl contained only ^{3}H radioactivity. The third fraction (C) eluted with 6 M urea, 2.0 M NaCl contained both ^{3}H and ^{35}S radioactivity.

Cellulose Acetate Electrophoresis: The P1 and P2 fractions obtained by gel filtration column chromatography (Fig 2) and the B and C fractions obtained by ion exchange column chromatography (results not shown) were analyzed by cellulose acetate electrophoresis. The radioactivity in each band was determined as described in *Materials and Methods* and the distribution of the cpm

Table II. Distribution of Labeled Constituents in Medium, Trypsinate, and Cell Residue

	Medium	Trypsinate	Cell Residue
% cpm ^{3}H			
+ PDGF	62 + 11	13 + 9	17 + 12
− PDGF	62 + 7	20 + 4	18 + 5
% cpm ^{35}S			
+ PDGF	47 + 9	27 + 7	26 + 6
− PDGF	51 + 7	23 + 12	22 + 4

Fibroblasts were incubated in the presence or absence of platelet-derived growth factor (PDGF). Each fraction was harvested, dialyzed, freeze-dried, and digested with pronase as described in *Materials and Methods*. Aliquots were taken from samples prior to column chromatography. The results are given in percent cpm ^{3}H and cpm ^{35}S incorporated into each fraction ± SD. This is the cumulative results of 5 hypertrophic cell lines.

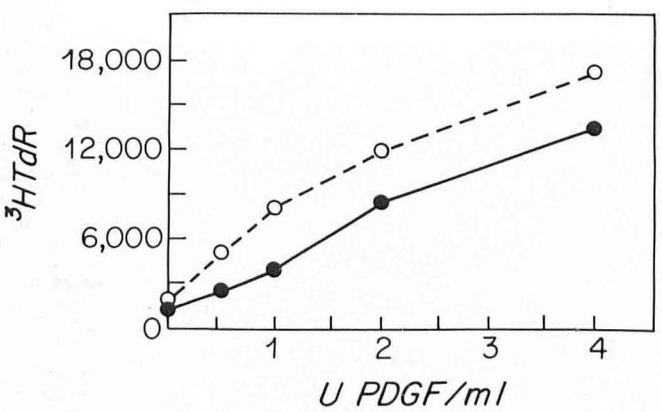

Figure 1. The PDGF-dependent uptake of [^{3}H]Thd by human scar fibroblasts. Confluent monolayer cultures of normal scar (*dotted line*) and hypertrophic scar (*solid line*) were incubated for 20 h in DMEM + 2% NuSerum + PDGF as indicated, pulse labeled with [^{3}H]Thd for 4 h, solubilized, and measured for cpm ^{3}H. Each point is an average of 6 determinations.

Table III. Decrease of ^{35}S Incorporation by Human Dermal Fibroblasts in the Presence of Platelet-Derived Growth Factor (PDGF)

Cell Line	Percent of Control	N
Nondialyzable material		
3493 NSc	92	5
2439 HSc	69	1
2266 HSc	52	1
CPC-ETOH precipitable material		
603 NSk	93	3
1865 HSc	92	3
3382 HSc	64	3

Confluent cultures of fibroblasts were incubated in the absence (control) or presence of 1 unit PDGF for 24 h. The conditioned medium was either (1) dialyzed and freeze-dried or (2) treated with pronase, and precipitated with cetylpyridinium chloride and then ETOH. The cpm ^{35}S in the resulting pellets were determined by liquid scintillation spectroscopy. The background cpm $^{35}SO_4$ from labeled nonconditioned medium has been subtracted and the results are expressed as cpm per 10^5 cells. N = number of determinations; NSc = normal scar; HSc = hypertrophic scar; CPC = cetylpyridinium chloride; NSk = normal skin.

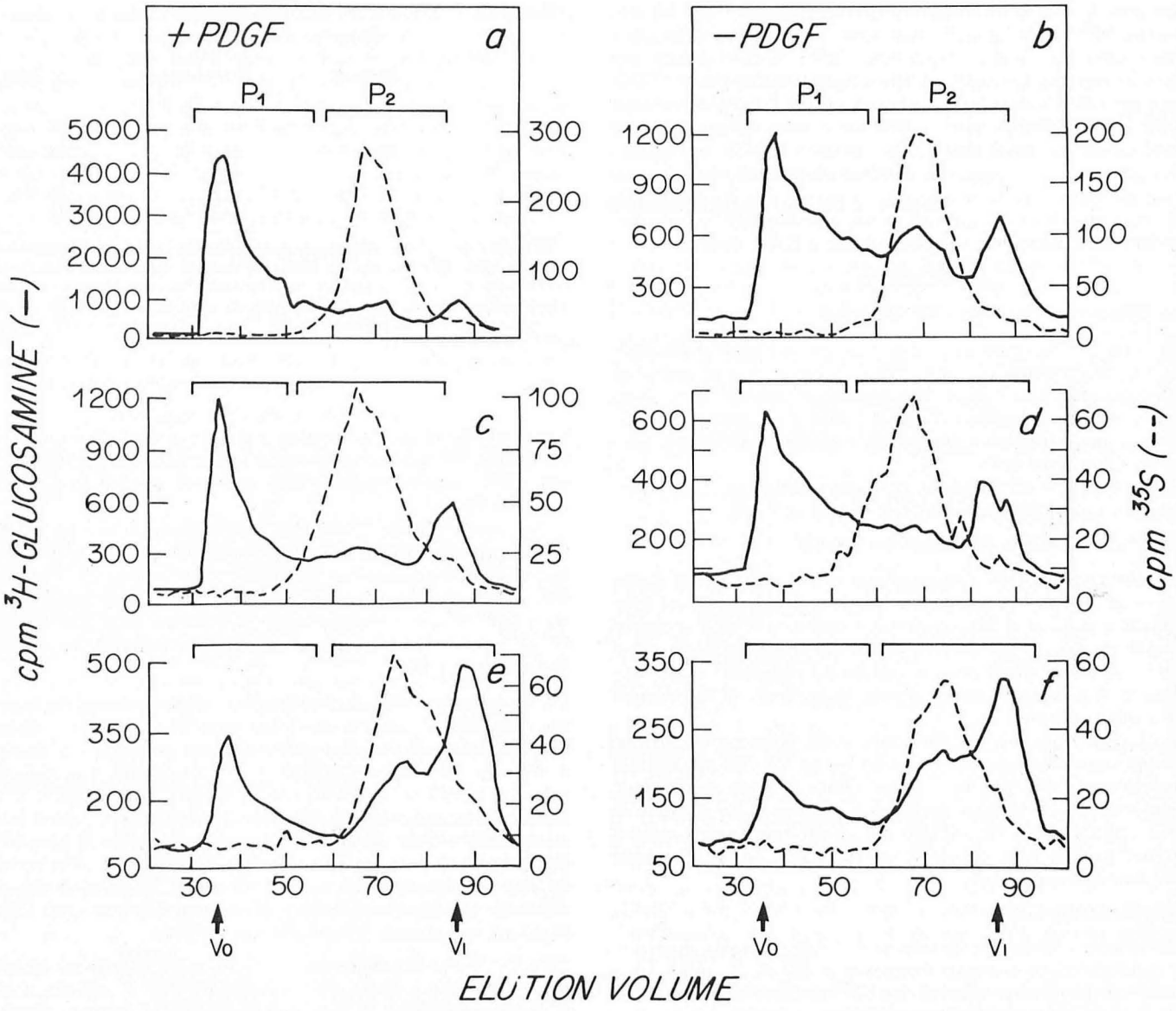

Figure 2. Column chromatography of the GAGs synthesized by normal skin fibroblasts in the presence and absence of PDGF. The medium (M) (*a,b*), trypsinate (T) (*c,d*) and cell (C) (*e,f*) fractions were collected from normal skin fibroblasts incubated in the presence (*a,c,e*) or absence (*b,d,f*) of 1 unit PDGF + 5 μCi [^{3}H]-glucosamine + 10 μCi ^{35}S/ml DMEM + 0.1% NuSerum for 24 h as described in *Materials and Methods*. Each sample was concentrated, treated with pronase, and then chromatographed on Sepharose CL4B column (1.5 × 44 cm) in 3 M guanidinium hydrochloride at a flow of 12 ml/h.

^{3}H and ^{35}S in the M, T, and C fractions are shown in Tables IV and V.

For all the samples chromatographed on the Sepharose CL4B column, the material eluting at the Vo (P1) labeled almost exclusively with [^{3}H]glucosamine and migrated as a single lobular band with the same mobility as the HA reference band (Fig 3). This band was eliminated by hyaluronidase treatment.

The material eluted in the CL4B column, P2 fraction, consistently contained 3 visible bands. One component with a similar mobility to the HA reference band was labeled with both ^{3}H and

Table IV. Incorporation of [^{3}H]Glucosamine Into Hyaluronate (HA) by Fibroblasts Cultured in the Presence and Absence of Platelet-Derived Growth Factor (PDGF)

	Medium		Trypsinate		Cell Residue	
	+PDGF	−PDGF	+PDGF	−PDGF	+PDGF	−PDGF
Normal skin	87	72	90	87	45	48
Hypertrophic scar	89	62	24	42	48	35

All samples were harvested, dialyzed, freeze-dried, and treated with pronase. Normal skin samples were fractionated by molecular sieve on Sepharose CL-4B and hypertrophic scar samples by ion exchange chromatography on DE-52. The results are given in the % cpm ^{3}H that migrates by electrophoresis on cellulose acetate with reference HA. The numbers are the average of duplicate determinations performed on fractions from single cell lines.

Table V. Distribution of cpm ^{35}S Among the Various Glycosaminoglycans (GAGs)

	Medium		Trypsinate		Cell Residue	
	+PDGF	−PDGF	+PDGF	−PDGF	+PDGF	−PDGF
Normal skin						
HP	11	17	19	23	25	25
DS	36	53	12	15	37	25
HS	4	7	44	30	21	32
DS′	29	10	5	14	8	10
CS	20	14	21	17	9	8
Hypertrophic scar						
HP	11	4	25	27	5	28
DS	<1	62	<2	2	4	10
HS	14	15	48	10	54	45
DS′	52	6	9	36	24	11
CS	23	7	16	15	12	4

The three fractions were harvested, dialyzed, freeze-dried, treated with pronase, and fractionated by column chromatography. The normal skin GAG fractions were isolated by CL-4B gel chromatography, while the hypertrophic scar GAG fractions were isolated by chromatography on DEAE cellulose. The individual GAGs were separated and identifieid by electrophoresis on cellulose acetate plates. Each band was cut out and the radioactivity measured by liquid scintillation. The results are given as % cpm ^{35}S migrating with the reference GAGs. The numbers are the average of duplicate determinations performed on fractions from single cell lines. HP = heparin; DS = dermatan sulfate; HS = heparan sulfate; DS′ = dermatan sulfate prime; CS = chondroitin sulfate.

^{35}S. Previously we have shown [19] that some of the material in this band is susceptible to hyaluronidase and the remaining radioactivity is digested by chondroitinase ABC but not by chondroitinase AC. This band of material resistant to hyaluronidase digestion is referred to as dermatan sulfate ′ (DS′). Another band which is clearly visible migrates with the dermatan sulfate (DS) standard, is also susceptible to chondroitinase ABC and is referred to as DS. A third band which is only faintly visible, migrates just slower than the C6S reference and is susceptible to both chondroitinase ABC and AC. An occasional band was observed with the same mobility as HP.

Analysis of the fractions obtained by the DEAE cellulose chromatography showed that the fraction (B) eluted with 6 M urea, 0.2 M NaCl contained only ^{3}H radioactivity and a band of material with the same mobility as the HA reference. The fraction (C) eluted with 6 M urea, 2.0 M NaCl contained both ^{3}H and ^{35}S radioactivity and the same GAG constituents present in the CL4B P2 fraction were detected.

Figure 4 shows the results obtained after treatment of the CPC and ethanol precipitate fractions with hyaluronidase. The 0.2 M NaCl, 1% CPC insoluble fraction contains visible constituents that have the same mobility as the CS, HA, and DS reference GAGs (lane 2). The band migrating with the HA reference was digested with hyaluronidase (lane 3). All of the bands were digested with chondroitinase ABC. Chondroitinase AC digested the band migrating with CS and reduced, but did not eliminate, the band migrating with HA (data not shown). Only one band, the one that migrated with the same mobility as the HA reference (lane 4) and was digested with hyaluronidase (lane 5), was isolated from the 0.2 M NaCl, 1% CPC soluble fractions.

There was an increase in the percent ^{3}H cpm incorporated into HA in the presence of PDGF (Table IV) in the M fraction from a normal skin fibroblast culture. For a HSc culture there was an increase in the percent cpm ^{3}H incorporated into HA in the M and C fractions and a major decrease in the trypsinate HA band in the presence of PDGF.

There were also differences in the distribution of ^{35}S-labeled sulfated GAGs obtained from fibroblasts cultured in the presence and absence of PDGF (Table V). In the medium fraction of both the normal skin and hypertrophic scar fibroblast cultures, the percentage of cpm ^{35}S incorporated into DS′ in the presence of PDGF increased while incorporation into the DS decreased. In addition, the percentages of cpm ^{35}S incorporated into CS was higher in all samples. In the trypsinate, of both normal skin and hypertrophic scar, fibroblast cultures treated with PDGF showed an increase in the percentage of cpm ^{35}S incorporated into heparan

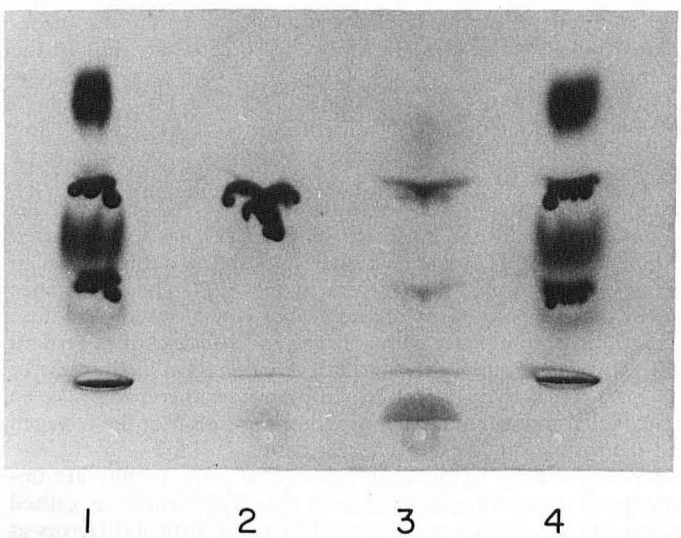

Figure 3. Cellulose acetate electrophoresis of GAGs synthesized by human skin fibroblasts in the presence of PDGF. *Lanes 1 and 4,* reference GAGs, origin (*OR*), heparin (*HP*), dermatan sulfate (*DS*), heparan sulfate (*HS*), hyaluronic acid (*HA*) also indicates where DS′ migrates, and chondroitin-6-sulfate (*CS*). *Lane 2* is P1 and *lane 3* is P2 from Fig 2*a*, medium conditioned by normal skin fibroblasts in the presence of 1 unit PDGF/ml DMEM + 0.1% NuSerum and fractionated on CL4B Sepharose.

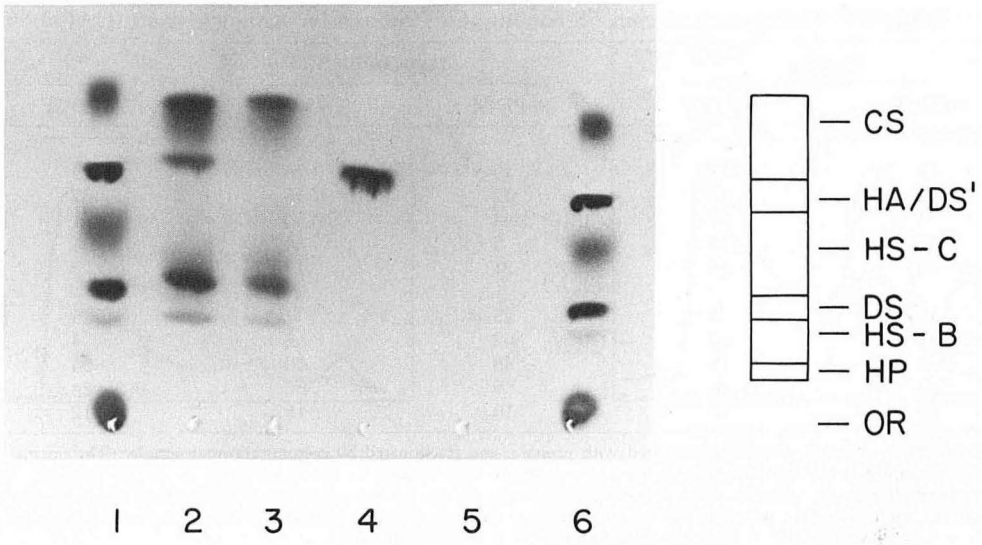

Figure 4. Hyaluronidase digestion of GAGs isolated from the conditioned medium of human scar fibroblasts. Medium conditioned by hypertrophic scar fibroblasts was digested with pronase, precipitated with 1% CPC. Hyaluronic acid was separated from the S-GAGs by its solubility in 0.2 M NaCl, 1% CPC, and all the GAGs were collected by precipitation in ethanol as described in *Materials and Methods. Lanes 1 and 6,* reference GAGs, dermatan sulfate (DS), heparan sulfate (HS-C, HS-B), hyaluronic acid (HA) and chondroitin-6-sulfate (*CS*); *lane 2,* 1% CPC, 0.2 M NaCl precipitable GAGs; *lane 3,* 1% CPC, 0.2 M NaCl precipitable GAGs digested in hyaluronidase (streptomyces) *lane 4,* 1% CPC. 0.2 M NaCl soluble GAGs; *lane 5,* 1% CPC, 0.2 M NaCl soluble GAGs digested with hyaluronidase. The plates were cut up as indicated and the quantities of radioactivity determined as described in *Materials and Methods. OR* is the origin. DS' is defined in the results section.

sulfate (HS). The HS values were calculated from the quantities of radioactivity in the heparan sulfate fraction B (HS-B) and heparan sulfate fraction C (HS-C) bands (Fig 4).

DISCUSSION

Our interest in PDGF is its potential for involvement in wound repair; it has been suggested that PDGF functions as a wound hormone [10]. Based on its in vitro chemotactic and mitogenic effects on fibroblasts, Seppa et al proposed that PDGF may help to populate the wound area with fibroblasts that synthesize the scar matrix. The purpose of the present experiments was to determine whether PDGF had a similar mitogenic effect on skin and scar fibroblasts and whether PDGF influenced the synthesis of GAGs. Differences in the composition of the GAGs in the extracellular matrices of skin and scar tissues have been noted [15–17] and it is thought that the altered GAG and PG composition of hypertrophic scars may contribute to the abnormal arrangement of collagen in these tissues.

Confluent cell cultures were used in these studies because we felt they more closely reflect in vivo state than exponentially dividing cell cultures. In order to discern the effects of PDGF on fibroblasts in vitro it was necessary to decrease the serum concentration from 10% used previously [19] to 1 or 2%. The differences observed in the distribution of ^{35}S into each of the sulfated GAGs and the distribution of ^{3}H and ^{35}S among the M, T, and C fractions from skin and scar fibroblasts in the present study (Table V) from those reported earlier [19] are thought to be due to differences in the serum concentration employed. Vogel and Sapien [22] have also found differences in the distribution of ^{35}S among the sulfated GAGs between human lung fibroblasts cultured in Eagle's minimal essential medium supplemented with high and low concentrations of newborn bovine serum.

Under the conditions used in the present study, we found that normal skin fibroblasts were more sensitive to the mitogenic effects of PDGF than scar-derived fibroblasts. In addition, we found that medium conditioned in the presence of PDGF contained a greater proportion of ^{35}S incorporated into DS' and a greater proportion of [^{3}H]glucosamine incorporated into HA than did medium conditioned in the absence of PDGF. A greater proportion of ^{35}S incorporated into HS was recovered from the cell trypsinate in the presence of PDGF.

That PDGF is mitogenic for fibroblasts [4–6,23] as well as confluent fibroblasts [3,7] is well documented and, in general, factors that stimulate cell division inhibit S-GAG synthesis. Therefore, it is to be expected that PDGF would decrease ^{35}S incorporation (Table III). Choi et al [24] reported that a factor isolated from human platelet lysate stimulated DNA synthesis and inhibited sulfated GAG synthesis by rabbit articular cartilage in monolayer culture. Prins and associates [25] also reported a decrease in ^{35}S incorporation into conditioned medium of rabbit articular chondrocytes in the presence of PDGF in medium supplemented with 1% or 10% heat-inactivated fetal bovine serum. A factor isolated from platelets, CTAP-III [26], stimulated both DNA synthesis as well as GAG synthesis. CTAP-III is also different from PDGF in that it has a M_r of 9,325 [27] compared with an estimated M_r of 33,000 for PDGF [26]. Several changes were noted in GAG synthesis in the presence of PDGF. There was an increase in the percentage of [^{3}H]glucosamine into HA (Table IV). This is consistent with the view that there are analogies between wound healing and development [28] and that HA synthesis and an HA-rich extracellular matrix is associated with regeneration and development [29,30].

In addition to increasing the percent [^{3}H]glucosamine incorporated into extracellular HA, PDGF also caused a shift in the synthesis of DS to DS' (Table V). In order to measure the synthesis of the various GAG constituents, we found it necessary to separate the sulfated GAGs from the more abundant HA. Ion exchange and molecular sieve chromatography were found to be equally effective in separating HA from the sulfated GAGs. Dermatan sulfate prime is a presumptive sulfated GAG that migrates with HA on cellulose acetate electrophoresis and has been reported in hog kidney [21], human dermal skin and scar fibroblasts in vitro [19], and human skin and scar tissues [31]. The importance of this shift in synthesis is unknown but it is of interest because DS is thought to interact with collagen and affect its fibril growth [32] and mechanical properties [33]. An alteration in the type of DS synthesized by cells in hypertrophic scar, therefore, may be a factor that influences the abnormal organization of the collagen and the formation of excessive scar tissue.

The controlling factors that lead to scar hypertrophy are unknown. Our working hypothesis is that hypertrophy is caused by the fibroblasts that are activated by environmental factors as

a result of injury and then fail to "deactivate" once the wound healing process is complete as occurs in normal scar formation. As a result, the fibroblasts responsible for scar hypertrophy continue to function in the activated state leading to the overabundant scar tissue characteristic of hypertrophic scars. Keloid fibroblasts synthesize excessive quantities of collagen compared with normal skin fibroblasts [34] and hypertrophic scar fibroblasts synthesize proportionately more CS and less HS [19] and more fibronectin [18] than normal skin fibroblasts. Our reported finding that normal skin fibroblasts are more responsive to PDGF than are scar fibroblasts fits in well with the notion that the hypertrophic scar fibroblasts are already in an activated state and thus less responsive to this mitogenic factor. The effects of PDGF reported resemble those of dedifferentiation, e.g., mitogenesis, increased HA synthesis, and decreased sulfated GAG synthesis, and are consistent with the early responses in wound healing. It appears possible, therefore, that PDGF may affect scar formation by increasing the population of fibroblasts and altering the type and amount of matrix components synthesized in the early stages of wound healing.

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