The Hypertrophic Scar. Hexosamine Containing Components of Burn Scars (36182)

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A recent study on the glycoprotein and collagen components of burn scars reported that hexose, hexosamine, sialic acid, uronic acid and glycogen levels were significantly increased in hypertrophic burn scars relative to either mature scars or to normal skin (1). This communication deals with further investigation of the hexosamine containing components of burn scars and normal skin.

Materials and Methods. Burn scar tissues removed for reconstructive purposes were clinically graded as hypertrophic or nonhypertrophic at the time of surgery. This evaluation was later verified by histological examination of a portion of the tissue. Patients involved were between 2 and 16 years of age; normal skin samples were obtained from the morgue of John Sealy Hospital from subjects between 6 and 82 years of age. All tissues were stored in the deep freeze until further processed. Fat and other subcutaneous tissue was removed from the samples with scissors; the samples were cut into thin strips, placed in drying bottles, frozen and dried from the frozen state. The dry tissues were ground in a Wiley Mill to pass through a 40 mesh screen.

Samples for glucosamine and galactosamine analysis, weighing approximately 100 mg were hydrolyzed in 4 ml of 4 *N* HCl in screw-capped test tubes at 100° for 16 hr. The hydrolyzed mixture was filtered through Whatman #40 paper and evaporated to dryness by means of an air jet. The dry samples dissolved in 1 ml of citrate buffer, pH 2.2, and 0.5 ml aliquots (representing approximately 50 mg of tissue) were placed on the medium column of the Beckman Amino Acid Analyzer. The column was packed with Aminex A-5 resin (BioRad Laboratories). The column was eluted with citrate buffer, pH 5.28. A calibration standard containing 0.2 μmol each hexosamine was run each day.

In order to identify the mucopolysaccharides found in the tissues, samples of approximately 100 mg were digested with papain (Sigma) by the method of Mier and Wood (2). After digestion the samples were dialyzed against tap water and freeze dried. The dried samples were dissolved in 1 ml of water and this solution used for cellulose acetate electrophoresis using the Beckman Microzone equipment. Electrophoresis was carried out with the zinc sulfate electrolyte (0.2 *M*, pH 5.1) of Breen *et al.* (3) with the calcium acetate electrolyte (0.3 *M* pH 7.25) of Seno *et al.* and with 0.25 *M* zinc sulfate. The 0.2 *M* zinc sulfate electrolyte separates hyaluronic acid, dermatan sulfate (chondroitin sulfate B) and chondroitin 4- or 6-sulfate (chondroitin A or C), but will not separate chondroitin-4-sulfate from chondroitin-6-sulfate. Calcium acetate and 0.25 *M* zinc sulfate will separate the 4-sulfate from the 6; however, the reaction of hyaluronic acid with Alcian Blue is inhibited in the stronger zinc sulfate and the separation in calcium acetate requires a longer electrophoretic time. As no chondroitin-6-sulfate could be demonstrated in any of the skin or scar samples, the 0.2 *M* zinc sulfate was used for quantitative work.

All electrophoretic strips were stained for 10 min with Alcian Blue (1.0% in 5% acetic

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acid), washed repeatedly with 5% acetic acid and quantitated without clearing on the microzone densitometer.

A standard containing equimolar amounts of chondroitin-4-sulfate, dermatan sulfate and hyaluronic acid was included in each electrophoretic strip. The densitometer readings for the two standard chondroitin sulfate bands were essentially the same, but those for hyaluronic acid were always less and varied from 50–65% of the chondroitin sulfate figures. The hyaluronic acid values obtained for the skin and scar samples were corrected accordingly for each run. The relative amounts of mucopolysaccharides were then calculated on the assumption that only hyaluronic acid, dermatan sulfate and chondroitin-4-sulfate were present. Absolute amounts of these mucopolysaccharides were calculated from the relative values and the total uronic acid. A correction was made in the total uronic acid figures obtained by the Bitter-Muir method (5) for the dermatan sulfate which gives only 80% of the color yield of chondroitin-4-sulfate.

Results and Discussion. A photograph of microzone electrophoresis patterns of mucopolysaccharides of scar and normal skin samples is shown in Fig. 1. Material with the approximate mobility of hyaluronic acid and dermatan sulfate is found in all samples. A definite third band with the approximate mobility of chondroitin-4-sulfate is present in hypertrophic scar samples. In a buffer system which separates chondroitin-4-sulfate and chondroitin-6-sulfate, nothing with the mobility of chondroitin-6-sulfate was detected. The presence of chondroitin-4-sulfate was further verified by treating samples with chondroitinase AC, chondroitinase ABC and hyaluronidase as described by Saito *et al.* (6). Photographs of the cellulose acetate electrophoretic patterns of the samples resulting from this treatment are shown in Fig. 2. After treatment of the samples with chondroitinase AC, the material with mobility of chondroitin-4-sulfate found in the hypertrophic scar largely disappears; after chondroitinase ABC the areas with the mobility of chondroitin-4-sulfate and dermatan sulfate disappear, but a band with the mobility of hyaluronic acid remains. Careful scanning of the hypertrophic scar patterns of material left after treatment with chondroitinase ABC also revealed two bands with approximately the mobility of heparitin monosulfate and heparin.

Results of the analytical work are summarized in Table I. A striking increase of galactosamine relative to glucosamine occurs in hypertrophic scars. This indicates an increase in the chondroitin sulfates in hypertrophic scars. A small, but statistically significant increase is present in galactosamine content of the nonhypertrophic scar when compared to normal skin.

The striking change in the mucopolysaccharides is the presence of a considerable amount of chondroitin-4-sulfate (chondroitin sulfate A). Individual values vary between 16% and 41% of the total mucopolysaccharide. By comparison the highest value for

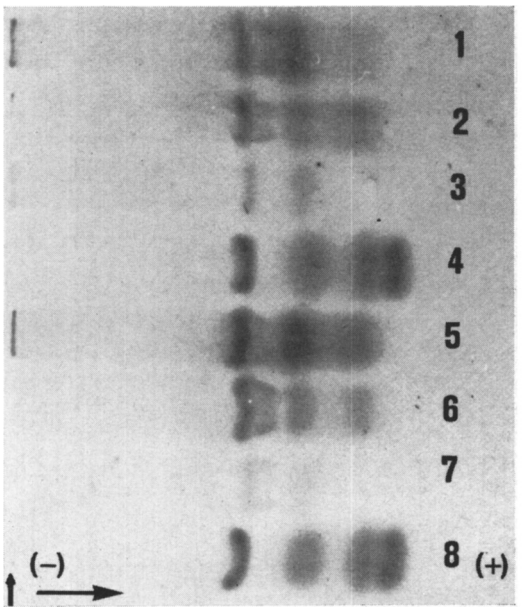

FIG. 1. Cellulose acetate electrophoresis patterns of mucopolysaccharides from scar tissue and normal skin. Electrolyte: 0.3 *M* calcium acetate. The origin is indicated by the small arrow and the direction of mobility by the large arrow. 4, 8. Standards containing hyaluronic acid (closest to origin), dermatan sulfate, chondroitin-4-sulfate and chondroitin-6-sulfate (to the right). 1. Nonhypertrophic scar. 2, 5, 6. Hypertrophic scars. 3, 7. Normal skin mucopolysaccharides.

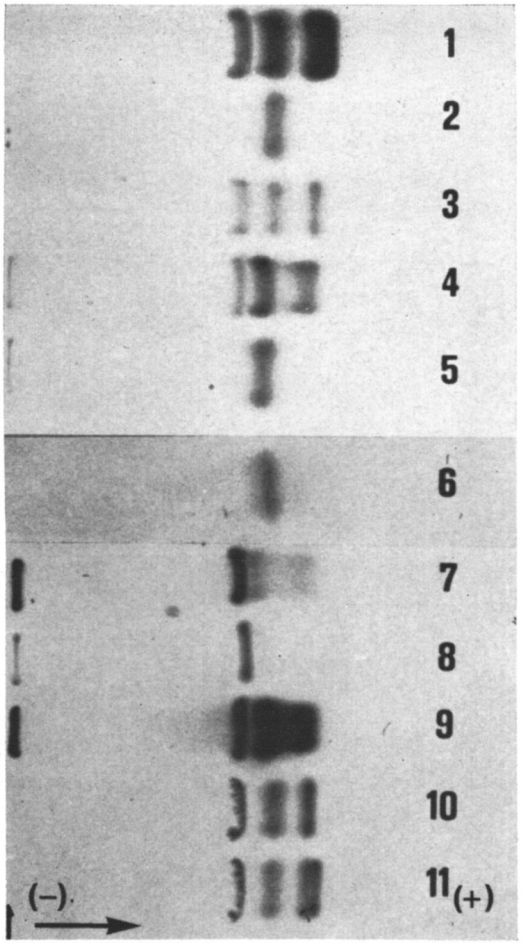

FIG. 2. Cellulose acetate electrophoretic patterns of mucopolysaccharides from scar tissue and skin before and after treatment with various enzymes. Electrolyte: 0.2 *M* zinc sulfate. The origin is indicated by the small arrow and the direction of mobility by the large arrow. 1, 11. Standard containing hyaluronic acid (band closest to origin), dermatan sulfate (middle band) and chondroitin-4-sulfate and -6-sulfate (not completely separated, to the right). 2. Standard similar to #1 after treatment with with hyaluronidase. Only dermatan sulfate remains. 3, 10. Standard containing hyaluronic acid, dermatan sulfate, and chondroitin-4-sulfate. 4, 9. Hypertrophic scar tissues without treatment. 5. Same preparation as No. 4 after treatment with hyaluronidase leaving the dermatan sulfate band. 6. Hypertrophic scar tissue (No. 4) after treatment with chondroitinase AC, only a dermatan sulfate band remains. 7. Hypertrophic scar tissue (No. 9) after treatment with chondroitinase ABC; a strong hyaluronic acid band remains, diffuse bands with the mobility of heparitin sulfate and heparin are also present. 8. Normal skin sample after treatment with chondroitinase ABC; only hyaluronic acid remains.

chondroitin-4-sulfate found in nonhypertrophic scar tissue was 11% and in normal skin, 7%. The significance of these findings is not clear. Loewi and Meyer (7) have reported that hyaluronic acid and chondroitin-6-sulfate (chondroitin sulfate C) are relatively high and dermatan sulfate is relatively low in embryonic skin. Granulation tissue obtained in the viscose cellulose sponge model has been reported to contain largely chondroitin-4-sulfate and hyaluronic acid; dermatan sulfate was not detected (8). If the absolute amounts of the mucopolysaccharides are considered, hyaluronic acid is essentially the same in hypertrophic scar tissue as in normal skin, but decreased in the nonhypertrophic scar. Dermatan sulfate, on the other hand, is increased in both hypertrophic and nonhypertrophic scar tissue. The hypertrophic scar has an increased amount of chondroitin-4-sulfate. The finding of this mucopolysaccharide in hypertrophic scars leads to interesting speculation. If this compound is present in recently formed connective tissue, its presence in the scar tissue is a suggestion that the tissue is actively forming new connective tissue. One may entertain the possibility that this mucopolysaccharide is involved in the formation of the hypertrophic scar either as an agent causing increased collagen biosynthesis or as an indication of failure of factors controlling connective tissue biosynthesis. Another possibility is that chondroitin-4-sulfate and dermatan sulfate are originally attached to the same protein core and that most of the chondroitin-4-sulfate is enzymatically removed as the tissue matures. A deficiency of the enzymes involved in this degradation may exist in hypertrophic scar tissue.

Summary. A study has been made of the glucosamine, galactosamine, hyaluronic acid and chondroitin sulfate composition of hypertrophic scars, nonhypertrophic scars and normal skin. The galactosamine was consistently and significantly elevated in hypertrophic scars, indicating that the chondroitin sulfates

TABLE I. Summary of Hexosamine Components of Hypertrophic Scars, Nonhypertrophic Scars and Normal Skin.

Component	Hypertrophic Scar (9)[e]	Nonhypertrophic Scar (8)	Normal Skin (9)
Glucosamine[a]	56.7 ± 1.9	75.9 ± 1.8	81.3 ± 1.2
Galactosamine[a]	43.3 ± 1.9	24.1 ± 1.8	18.7 ± 1.2
Hyaluronic acid[b]	18.5 ± 0.8	21.0 ± 1.5	41.5 ± 2.7
Dermatan sulfate[b]	55.1 ± 3.0	70.5 ± 1.6	54.0 ± 2.9
Chondroitin-4-sulfate[b]	26.4 ± 2.9	8.5 ± 1.3	4.5 ± 0.7
Total uronic acid[c]	1.83 ± .11	0.93 ± .03	0.83 ± .04
Hyaluronic acid[d]	0.71	0.47	0.82
Dermatan sulfate[d]	2.32	1.72	1.07
Chondroitan-4-sulfate[d]	1.31	0.19	0.09

[a] Expressed as a percentage of the total hexosamine.

[b] Expressed as a percentage of the total mucopolysaccharide.

[c] Expressed as mg/g of dry tissue.

[d] Calculated by multiplying uronic acid values × 2.37 × the respective percentage of the mucopolysaccharide.

[e] Results given are averages of 9 hypertrophic scars, 8 nonhypertrophic scars and 9 normal skin samples. Figures following ± sign are standard errors of the mean.

were increased. Dermatan sulfate was found to be elevated in both hypertrophic and nonhypertrophic scars. Chondroitin-4-sulfate was found in the hypertrophic scar in significant amounts; only traces of this mucopolysaccharide were found in nonhypertrophic scar and in normal skin.

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Received Oct. 13, 1971. P.S.E.B.M., 1972, Vol. 139.