The Hypertrophic Scar. Glycoprotein and Collagen Components of Burn Scars (35883)

M. R. Shetlar, Mario Dobrkovsky, Hugo Linares, Rebecca Villarante, C. L. Shetlar, and Duane L. Larson

Departments of Biochemistry and Surgery and the Shriners Burns Institute, University of Texas Medical Branch, Galveston, Texas 77550

The interrelationships between collagen, mucopolysaccharides, and glycoproteins are considered to be of importance in wound healing because of the postulated role of glycoproteins and mucopolysaccharides in the stabilization of the collagen fiber. An increase of connective tissue occurs locally in the hypertrophic scar which results from burn injury. However, it is not clear whether this is due to an increase in biosynthesis or to a decrease in the rate of breakdown. If increased biosynthesis occurs, one would expect an increase of glycoproteins and mucopolysaccharides which is typical of proliferating connective tissue (1). Some insight into the glycoprotein, mucopolysaccharide, and collagen relationships of a tissue may be obtained by analysis of the carbohydrate constituents and the collagen fractions. Complete studies of carbohydrate constituents have not been made in human skin although Herp and Pigman (2) have reported a study of rat skin. The following describes a comparative study of hypertrophic burn scar tissue nonhypertrophic scar tissue, and normal human skin.

Materials and Methods. Burn scar tissues removed for reconstructive purposes were clinically graded as hypertrophic or nonhypertrophic at the time of surgery. Patients involved were between 2 and 16 years of age. Normal skin samples were obtained from the morgue of John Sealy Hospital; ages of individuals varied between 6 and 82, however, the majority were adults. All tissues were stored in the deep freeze in closed jars until further processed. Fat and other subcutaneous tissue were removed from the scar or skin with scissors; the tissue was cut into thin strips, placed in drying bottles, frozen, and dried from the frozen state. The dried tissues were ground in a Wiley mill to pass through a 40-mesh screen.

Hexose was determined on the dry tissue by the tryptophan method (3). Hexosamine was determined by the method of Cessi and Piliego (4). Uronic acid was determined with the carbozole method as modified by Bitter and Muir (5) after hydrolysis using Dowex 50 as a catalyst (6); a correction was made for the hexose content of the hydrolysate. Glycogen was determined by a method modified from that of Seifter *et al.* (7). Sialic acid was determined by the thiobarbituric acid method of Warren (8). Salt-soluble collagen was determined by stirring 100 mg of the dried tissues for 24 hr with 10 ml of 0.2 *M* NaCl solution. The residue was separated from the supernatant by centrifugation at 12,000*g* for 30 min. The residue was again stirred for 24 hr with another 10 ml of 0.2 *M* NaCl. The mixture was centrifuged and the supernatant was added to the original one. This procedure was repeated one more time. The residue was similarly extracted with 0.2 *M* citrate solution, pH 3.5, and the supernatants were combined. Finally the residue from the citrate extraction was suspended in water and autoclaved at 15 lb pressure for 30 min to convert the insoluble collagen to gelatin. All collagen fractions were made to a definite volume; aliquots were taken for hydroxyproline determinations by the procedure of Bergman and Loxley (9).

Results and Discussion. Results of the analytical work are summarized in Table I. All carbohydrate components were significantly higher in the hypertrophic scars than in either nonhypertrophic scars or in normal skin. The nonhypertrophic scars had slightly, but

TABLE I. Summary of the Carbohydrate Constituents and Collagen Fractions of Hypertrophic Scars, Nonhypertrophic Scars, and Normal Skin.[a]

Component	(mg/g of dry tissue)		
	Hypertrophic scar	Nonhypertrophic scar	Normal skin
Hexose	17.7[b] ± 0.7	10.8[c] ± 0.1	9.8 ± 0.1
Hexosamine	6.34[b] ± 0.25	3.68[c] ± 0.18	2.68 ± 0.13
Sialic acid	1.84[b] ± 0.07	0.99[c] ± 0.04	0.80 ± 0.02
Uronic acid	1.60[b] ± 0.09	0.88[c] ± 0.03	0.72 ± 0.04
Glycogen	2.11[b] ± 0.21	1.37[c] ± 0.11	0.84 ± 0.10
Hydroxyproline of			
salt-soluble collagen	1.42[b] ± 0.10	0.91 ± 0.11	0.89 ± 0.12
citrate-soluble collagen	1.57 ± 0.06	1.51 ± 0.07	1.53 ± 0.08
insoluble collagen	59.51 ± 1.61	59.46 ± 1.76	62.48 ± 2.11

[a] Results given are averages of 14 hypertrophic scars, 15 nonhypertrophic scars and 12 normal skin samples; values ± standard errors of the mean.

[b] Significantly higher than the comparable values for both nonhypertrophic scar and normal skin at the 1% level of probability.

[c] Significantly higher than the values for normal skin at the 1% level of probability.

significantly, higher values of all carbohydrate constituents than did the normal skin. Since most of the normal skin samples were from adults, these differences may be due to age changes. Two normal samples from children (2 and 6 years of age) were both slightly higher than the normal averages in uronic acid and sialic acid content. The higher uronic acid contents indicate that the mucopolysaccharide content of hypertrophic scars is increased while the increased sialic acid indicates that the sialic acid-containing glycoproteins are also elevated. A similar conclusion was made by Bazin *et al.* (10), who studied uronic acid and sialic acid in scars. In our data, the hexosamine associated with glycoprotein may be estimated by determining the amount of hexosamine equivalent to the uronic acid and deducting this value from the total hexosamine values. The hexosamine associated with glycoprotein is as follows: hypertrophic scars, 4.86; nonhypertrophic scars, 2.81; and normal skin, 2.02 mg/g of dry tissue.

The chemical composition and the physiological role of these glycoproteins in scar and skin are of interest. Some of the glycoproteins of skin appear to be the same as plasma proteins (11), however, it is not known whether these are increased in hypertrophic scar tissue. Another estimate of glycoprotein may be calculated by deducting the hexose of glycogen and the hexose content of collagen from the total values; these calculated values are 13.8, 7.6, and 7.2 mg of hexose/g of dry tissue for hypertrophic scar, nonhypertrophic scar, and normal skin. The higher hexose levels of the hypertrophic scar again indicate higher glycoprotein levels. This may indicate that the distribution of proteins formed in hypertrophic scars are different or that these proteins have a higher hexose content.

Salt-soluble collagen of the hypertrophic scars was found to be higher than that of the nonhypertrophic scars and normal skin, as indicated by hydroxyproline contents of the fraction. Other collagen fractions were not found to differ significantly. These latter findings are similar to those reported by Sibeleva *et al.* (12), who determined total collagen content of keloids, normal scars, and normal skin and found no significant differences.

The presence of increased amounts of mucopolysaccharide and of increased salt-soluble collagen is characteristic of young or proliferating connective tissue. Consequently, it appears that the hypertrophic scar is more active metabolically than the nonhypertrophic scar or normal skin. Further investigation

is necessary to determine how hypertrophic scar differs biochemically from normally growing skin.

Summary. A study of the hexose hexosamine, sialic acid, uronic acid, glycogen, and collagen fractions has been made on hypertrophic scars, nonhypertrophic scars, and normal skin. Hexose, hexosamine, sialic acid, uronic acid, and glycogen levels were significantly and consistently elevated in hypertrophic scars. These same components were slightly, but significantly, higher in the nonhypertrophic scars. Salt-soluble collagen of hypertrophic scars was significantly elevated, citrate-soluble and insoluble collagen fractions were not different in the three groups. From these data it is concluded that hypertrophic scars contain more glycoprotein and mucopolysaccharide and are more active metabolically.

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Received May 13, 1971. P.S.E.B.M., 1971, Vol. 138.