Uronic Acid-Containing Polypeptides from the Urine of Burned Patients[1] (34354)

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Material containing glucosamine has been reported to be elevated in the urine of burned patients by Estes and Blocker (1). Estes (2) has also reported the presence of three peptide fractions isolated by resin chromatography in the urine of burned patients which do not occur in the urine of normal subjects. One of these peptide fractions contains appreciable amounts of glucosamine, hexose, uronic acid, and sialic acid. The increased excretion of glucosamine may be due to metabolic products of the elevated serum glycoproteins which occur after burn injury (3) or may be products of either tissue glycoproteins or tissue mucopolysaccharides. The uronic acid, on the other hand, if linked to peptide, should only be related to the mucopolysaccharide–protein complexes of tissue. To further investigate this question of the type of glycopeptides found in urine after burn injury, we have undertaken the following work concerning complexes which contain uronic acids.

Materials and Methods. Twenty-four-hr urine collections were made in the hospital. During the collection period the urine was kept refrigerated; thymol crystals were used as a preservative. At the end of the collection period, volumes of the samples were measured and the urines were stored in the deep freeze until further work was undertaken. The urines were then brought to room temperature; 250 ml were dialyzed against deionized water for 48 hr. The nondialyzed material was lyophilized and redissolved in 25 ml of deionized water. An aliquot (usually 2 ml) was used for uronic acid analysis using the Dowex hydrolysis described by Shetlar (4) followed by the carbazole procedure as modified by Bitter and Muir (5). Hexosamine content of the nondialyzible material was determined on another aliquot of the same concentrate using a modified method as previously described (4). Hexose content of the same material was determined by the tryptophane method (4).

The mucopolysaccharides were isolated by a modified procedure of Kao *et al.* (6). By this procedure the Tamm-Horsfall glycoprotein fraction was precipitated with 0.58 *M* NaCl. The supernatant was dialyzed and ammonium sulfate was added to it until full saturation was achieved. The precipitate contains hyaluronic acid-containing complexes and some glycoproteins. The supernatant from the $(NH_4)_2SO_4$ precipitation contains the chondroitin–sulfate complexes together with other glycoproteins. In order to further separate these compounds, the two fractions were subjected to cellulose acetate electrophoresis using a pyridine–acetic acid buffer (pH 3.4; 10 ml of pyridine, 100 ml of glacial acetic acid, add water to 1 liter). This buffer will satisfactorily separate chondroitin sulfates A and C from chondroitin sulfate B. The $(NH_4)_2SO_4$ precipitate was suspended in water and dialyzed against deionized water until free of sulfate as indicated by testing with $BaCl_2$ solution. The $(NH_4)_2SO_4$ supernatant was similarly dialyzed against deionized water until free of sulfate. Both solutions were then lyophilized; the total dry material was weighed and a known amount was weighed out, dissolved in water (usually 0.1–0.2 ml), and an aliquot (0.75–1.25 μl) was subjected to electrophoresis on the Beckman Microzone apparatus. Resulting patterns

[1] Supported in part by PHS Grant GM 10903.

TABLE I. Uronic Acid, Hexosamine, and Hexose Contents of Nondialyzable Urinary Components of Normal Subjects and Patients with Burn Injury.

Subject[a]	Sex	Postburn (days)	Excreted (mg/24 hr)		
			Uronic acid	Hexosamine	Hexose
L.S. (5–2°)	F	5	12	43	95
		12	13	36	84
A.W. (28–2°)	F	10	14	93	271
		22	32	80	240
G.A. (30–2°)	M	11	21	208	363
L.U. (32–2°)	M	3	74	170	334
		20	31	49	139
E.R. (40–2°)	M	9	22	149	371
G.H. (41–2°)	M	4	16	72	168
		6	15	68	147
Normals	M (3)	—	7–12	28–29	64–90
	F (2)	—	6–7	18–23	48–55

[a] Values in parentheses following patient's initials are percentage of body area burned followed by degree of burn, e.g., 28–2° indicates a second degree burn of 28% of the body surface.

were quantitated on the Beckman Microzone densitometer.

Results and Discussion. Results of the uronic acid, hexosamine, and hexose analyses of the nondialyzible portion of urines for 5 normal and 6 burned patients are summarized in Table I. Normal values are similar to those previously reported (4). Higher values of nondialyzable hexosamine were found after burn injury. This is in agreement with Estes and Blocker (1) who reported higher levels of total hexosamine after burns. Our hexosamine results are somewhat lower for burned patients than are theirs. This may indicate the presence of increased amounts of dialyzable glucosamine, either free or in small conjugates, following burn injury. The nondialyzable uronic acid excretions of patients with burn injury are decidedly increased over those of normal subjects. There may be some relationship to extent of injury in that one patient with only a 5% burn was only slightly higher than the highest normal subject. The hexosamine in normal subjects was in excess of the uronic acid levels (ratio approximately 3:1). This would appear to indicate that some of the bound hexosamine found in urine is derived from complexes other than the chondroitin sulfate and hyaluronic acid. Following burns, the hexosamine appears to increase relative to the uronic acid. Two samples, 20 and 22 days postburn, however, were found to have normal or decreased ratios of hexosamine to uronic acid. These observations confirm the suggestion that metabolism of a number of types of compounds contribute to the bound hexosamine of urine. A similar conclusion may be made from the hexose data of Table I. Metabolites of collagen and other connective tissue proteins may contribute to these values.

Cellulose acetate electrophoresis of the ammonium sulfate precipitated material followed by staining with alcian blue revealed the presence of material with the mobility of standard hyaluronic acid. This material, as well as the hyaluronic acid, failed to stain with toluidine blue. When quantitated, this fraction showed some tendency to increase after burn injury, but most values were within the normal range (Table II). In addition, material was found in this fraction which remained at the origin, but stained with toluidine blue and alcian blue. This indicates the possible presence of sulfated mucopolysaccharide bound to protein; these fractions also stained strongly for protein with ponceau S. The urine from burned patients contains considerably more material staining with alci-

TABLE II. Mucopolysaccharide Material in Urine of Normal Subjects and Patients with Burn Injury.

Subject[a]	Sex	Postburn (days)	Excreted (mg/24 hr)			
			Chondroitin sulfate			Hyaluronic acid
			Total	A	B	
J.M. (23–2°, 5–3°)	M	5	5.2	1.1	4.1	0.3
		9	14.9	4.7	10.3	5.5
		18	12.4	—	12.4	15.5
J.W. (27–2°)	M	5	19.1	8.6	10.5	1.2
		9	22.9	11.1	11.8	3.0
E.R. (40–2°)	M	2	13.6	0	13.6	0.8
		9	18.8	1.7	17.1	1.0
		15	47.0	7.0	40.0	1.6
J.F. (20–2°, 35–3°)	F	3	18.5	0	18.5	1.8
Normals (6)			6.2–10.9	3.7–10.9	0–4.1	0.5–7.3

[a] Values in parentheses following patient's initials are percentage of body area burned followed by degree of burn, e.g., 23–2° indicates a second degree burn of 23% of the body surface.

an blue (Table II) and most of the increase occurs in the material with the mobility of chondroitin sulfate B; this material increases with time after the injury. Much of this material appears to have a slower mobility than the standard chondroitin sulfate B (Fig. 1). When stained with toluidine blue much of this slower moving material failed to stain (Fig. 2). This may indicate that this material is not completely sulfated.

Summary. The peptides of urine containing uronic acids of burned and normal subjects were investigated by quantitative estimation of the uronic acid, hexosamine and hexose content of the nondialyzable urinary components. The daily excretions of nondialyzable uronic acid, hexosamine, and hexose were all increased after burn injury. The mucopolysaccharides of urine were further investigated by partial isolation with $(NH_4)_2SO_4$ followed by cellulose acetate electrophoresis. Material with the mobility and staining characteristics of hyaluronic acid, chondroitin sulfate A or C, and chon-

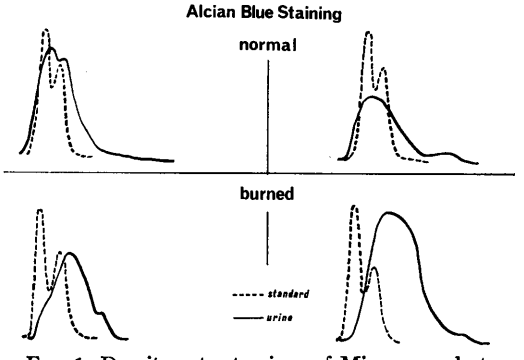

FIG. 1. Densitometer tracings of Microzone electrophoresis patterns of urinary material soluble in saturated ammonium sulfate solution. Patterns stained with alcian blue; (- - -), patterns of a standard solution of chondroitin sulfate A (to left) and chondroitin sulfate B (to right).

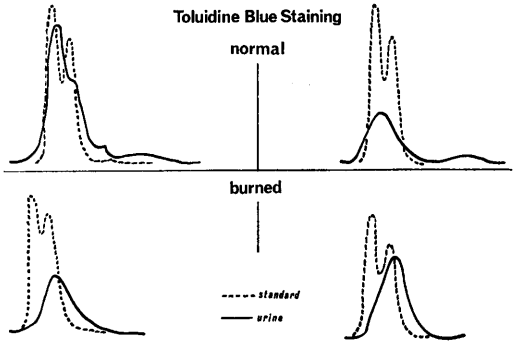

FIG. 2. Densitometer tracing of Microzone electrophoresis patterns of urinary material soluble in saturated ammonium sulfate solution. Patterns stained with toluidine blue; (- - -), patterns of chondroitin sulfate A (to left) and chondroitin sulfate B (to right). Samples used are the same shown in Fig. 1.

droitin sulfate B were found in urine from both normal and burned persons. Only the chondroitin sulfate B fraction was consistently elevated after burn injury. There are indications that some of this fraction is incompletely sulfated.

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Received July 16, 1969. P.S.E.B.M., 1969, Vol. 132.