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Urinary Hydroxyproline: Source of Increase After Thermal Burns[1] (37919)

J. GRAHAM SMITH, JR., RICHARD F. WEHR, NANCY L. BADGER, AND DONALD E. PIRKLE

Departments of Dermatology and Medicine, Medical College of Georgia, Augusta, Georgia 30902

Collagen makes up about 20–25% of the total mammalian body protein (1) and is characterized by the amino acid hydroxyproline (hypro) which constitutes about 14% of its amino acid residues (2).

Elastin, which is much less abundant than collagen, also contains small amounts of hypro (3), but no other mammalian protein contains a significant quantity. Free hypro is not incorporated into collagen and its precursor is proline (Pro) (4, 5).

Newly synthesized collagen is easily extracted from connective tissues with cold electrolyte solutions and has been referred to as soluble collagen (6). As the collagen matures or ages, it becomes progressively more polymerized and becomes insoluble to extraction with these solutions (7). In rats, approximately 1% of the collagen from skin or carcass is soluble in cold sodium chloride or dilute acid, and the remainder is insoluble (7, 8).

Urinary hypro excretion has been studied as an index of collagen metabolism because the final breakdown products of collagen include both free and peptide hypro which are not reutilized for collagen synthesis (9). Peptide-bound hypro makes up over 95% of the total urinary hypro with less than 5% being excreted as free hydroxyproline (10). In "young adult" rats, isotopic studies have shown that 90–95% of the degraded insoluble collagen is metabolized, and the 5–10% of the degraded insoluble collagen that is excreted as hypro in the urine makes up approximately two-thirds of the total urinary hypro, the remainder originating from newly synthesized soluble collagen (8).

Thus urinary hypro excretion is not a sensitive indicator of collagen metabolism due to the fact that extremely marked changes in collagen degradation are necessary to result in altered excretion.

Thermal burns produce very high levels of urinary peptide hypro excretion, and the purpose of this study was to determine the source of this increased urinary hypro. If the major source is from the urinary excretion of proteolytically degraded thermally denatured collagen from burned skin, total hypro in burned skin should decrease following the burn while there would be little significant change in the total hypro in the unburned intact skin and the carcass.

Methods. Eighteen female Sprague–Dawley rats (weighing 226.4 ± 3.6 g) were anesthetized with ether, and the entire dorsal surface was shaved with an electric clipper from the neck to the tail. Under anesthesia, the animals were immersed in 100° water for 15 sec so that the dorsal surface from the neck to the tail and half way over the sides of the body was immersed. Six animals were sacrificed immediately after receiving the thermal burn. Six animals were sacrificed at 8 and 16 days after burning.

After sacrifice, the animals were skinned, excluding the skin of the head and paws. The remainder of the hair was clipped. The burned skin was separated from the intact (unburned) skin by carefully cutting along the easily discernible junction between

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TABLE I. Total Collagen Hydroxyproline from Burn Skin, Intact Skin, and Carcass (mg/Total Specimen.[a]

	Days after burn		
	0	8	16
Burn skin	243.5 ± 5.8	188.2 ± 11.1*	156.7 ± 9.6*
Intact skin	128.4 ± 6.6	113.0 ± 6.7	117.5 ± 7.0
Carcass	557.2 ± 12.1	541.8 ± 23.7	543.0 ± 13.2
Newly formed connective tissue			51.5 ± 4.8

[a] Results expressed as mean ± SEM. $N = 6$ for each mean.
* $P < .005$.

burned and unburned skin and the underlying fat removed from both.

Each sample of burned skin and intact skin (with fat removed) was cut into small segments. The carcass along with the fat, which was removed from the burned and intact skin, was ground in a commercial meat grinder. All three separate specimens were then extracted for 30 min with hot (90°) 0.3 *M* trichloroacetic acid, the supernatant removed, and the insoluble material homogenized and then reextracted as above. The resulting pellet was washed three times with cold 5% TCA, with the supernatants being combined (11, 12). Two milliliter aliquots from the supernatants were then combined with 2 ml 12 *N* HCl and hydrolyzed without prior dialysis (12) for 16 hr and analyzed for hypro (13). In a separate experiment, 3 female Sprague–Dawley white rats were burned as outlined above and placed in metabolic cages. Twenty-four-hour urines were collected daily and analyzed for hydroxyproline.

Results. Because there is a 2–5 times greater amount of collagen hypro in the dorsal pelt of rats as compared with the ventral (14), the burns which covered about 35% of the dorsal surface area actually represent thermal injury to approximately 65% of the total skin collagen.

The loss of hypro from the burn skin (Table I) was statistically significantly lower ($P < .005$) by Day 8 as compared to Day 0 and continued to be so at Day 16. The decrease in collagen hypro in the intact skin at Days 8 and 16 as compared with Day 0 was not statistically significant.

There was no statistical loss of collagen hypro from the carcass, and by Day 16 a layer of newly formed connective tissue had formed under the burned tissue which contained a large amount of hypro (Table I).

As seen in Fig. 1, the urinary hydroxyproline excretion increased by a factor of 5.

Discussion. The statistically significant drop ($P < .005$) in collagen hypro in the burned skin at Days 8 and 16 supports the original hypotheses of Klein *et al.* (15), who first reported increased urinary peptide hypro following severe thermal burns, and suggested that these high levels of urinary hypro reflected proteolytic breakdown of thermally denatured dermal collagen. To support this further, Klein and Davis (16) found in 11 burned children a good correlation between urinary peptide hypro and percent total surface area burn ($r = 0.63$), percent third degree burn ($r = 0.53$), and the Brooke burn index ($r = 0.60$).

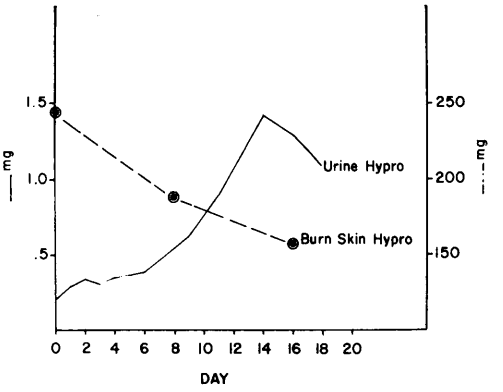

FIG. 1. Urinary hydroxyproline excretion concurrent with burn-skin hypro loss.

Kyuz and Oppel (17) reported an initial increase in hypro and a depression and then an increase of soluble collagen content in the skin during the 15 days postburn.

The skin becomes quite necrotic by Day 16, and cracks can appear or areas can be missing due to animal agitation. The Day-16 burned areas were determined and the area lost or missing averaged 4.9%. No general overall sloughing was observed. The weight of the burned area decreased by 15.6%, probably due to loss of water from the necrotic skin. In no case, however, can these conditions account for the 35.65% loss of hypro from Day 0 to Day 16.

In studies of urinary hypro in thermally burned rats whose collagen hypro was labeled with ^{3}H-proline (^{3}H-Pro) 3 weeks prior to burning and with ^{14}C-proline (^{14}C-Pro) the day of and for 4 days after burning, Jackson and Elliott (18) found that with the exception of the first day after burning there was significantly greater excretion of ^{3}H-Pro through Day 17 after the burn. The unburned animals excreted significantly more ^{14}C-hypro than the burned animals. Based on this work, Jackson and Elliott concluded that there was not stimulation of collagen synthesis by the burn through Day 17 and that the "generalized effect of a burn on collagen metabolism appears to be purely catabolic with no compensatory anabolic component."

In studies of human urinary *O*-hydroxylysl glycosides as an index of collagen metabolism, Segrist and Cunningham (19) found the highest levels in a single 65-year-old male 8 days after a 50% thermal burn. This patient's urinary ratio of hydroxylysine:galactose:glucose (hyl:gal:glu) to hydroxylysine:galactose (hyl:gal) was also higher than that of any other patients studied. Since they found the ratio of hyl:gal:glu to hyl:gal higher in adult skin than bone or cartilage, they concluded the ratio in this burn patient was a result of increased turnover in skin collagen as compared with other collagen including bone.

That the newly synthesized collagen may be contributing to the increased urinary peptide hypro by Day 16 is suggested by the finding of newly formed connective tissue under the burn site. Jackson and Elliott (18) stopped the administration of ^{14}C-Pro on Day 4 after the burn, and thus the increased synthesis by Day 16 would probably not have been detected.

A decrease in burned-skin hypro concurrent with an increase in urinary hydroxyproline with time has been demonstrated in thermally injured rats.

Summary. The collagen hydroxyproline (hypro) from burned skin, unburned intact skin, and carcass of thermally burned rats has been studied. The statistically significant decrease in burned skin hypro 8 and 16 days after burn suggests that the major source of urinary peptide hypro early after burns is from degradation of thermally denatured skin collagen. The finding of a new layer of hypro-rich connective tissue under the burn site on Day 16 suggests that later elevations of urinary hypro may result from breakdown products of newly synthesized collagen as well as degradation of collagen at the burn site.

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