EFFECT OF THERMAL INJURY ON EXCRETION OF HYDROXYPROLINE IN RATS, TREATED OR NOT WITH AN ANABOLIC ANDROGEN

By

K. Kowalewski and F. Heron

ABSTRACT

Urinary excretion of hydroxyproline, considered as an index of collagen metabolism, was determined prior to, and during, a period of healing of thermally injured rats' skin. Standard deep dermal burns (about 12% of the total skin surface) were produced in rats. Animals were left untreated or received, during the healing period, an anabolic steroid 17β-hydroxy-17α-methylandrostano [3,2-c] pyrazole (Winstrol®). Urine was collected prior to injury and after burns. Hydroxyproline was determined and expressed in μg per 24 h per 100 g of rats' body weight. Thermal injury affected significantly hydroxyproline excretion: progressive increase of this component of collagen was observed, with peak value in third week after burns. This was considered due mostly to the proteolysis of irreversibly denatured collagen. However, some excreted hydroxyproline might represent the product of newly formed collagen of healing tissue. Anabolic steroid used in this study produced an increase in hydroxyproline excretion as compared with untreated controls, but this effect was apparent only during the third week after injury. Hydroxyproline excretion test may be of value following thermal injury of the skin. Experimental results in this respect, found in rats, are similar to those found previously in humans.

Most studies on thermal injury are concerned with histological lesions or effects of burns on water, electrolytes and protein metabolism (Entin & Baxter 1950; Davis & Abbott 1956; Leap 1967). These effects depend upon the extent and depth of burns and upon the damage of skin protein by denaturation. Such denaturation affects particularly the skin collagen. Collagen, the most abundant protein of the skin, is the only one that contains hydroxyproline in significant amount. Because of this virtually unique distribution, hydroxyproline is ac-
cepted as an index of collagen (Laitinen 1967). We previously studied various fractions of hydroxyproline in healing burned skin of rats, and we described in detail the pattern of response of dermal collagen to thermal injury (Kowalewski & Yong 1969). Such an experiment on tissue collagen is not only a laborious one, but not easy under clinical conditions. Another more practical method of studying changes in body collagen, a method clinically feasible, is the determination of urinary hydroxyproline. Urinary excretion of hydroxyproline reflects collagen metabolism (Prockop & Sjoerdsma 1961; Jasin et al. 1962; Smiley & Ziff 1964). Elevated excretion of hydroxyproline has been found in the conditions associated with increased synthesis and/or with increased breakdown of collagen (Benoit et al. 1963; Kivirikko et al. 1963, 1964; Smiley & Ziff 1964; Jones et al. 1964; Kowalewski 1965; Kowalewski & Yong 1967). In patients with dermal burns, elevated excretion of hydroxyproline was also observed and was considered due to the proteolysis of irreversibly denatured collagen of injured skin (Klein et al. 1962; Klein & Davis 1964).

No study on urinary hydroxyproline in burned animals came to our attention, and the purpose of the present experiment was to explore this field in rats. Because it is known that some hormones affect collagen of normal and injured skin (Houck & Patel 1965; Laitinen 1967; Kowalewski & Yong 1968a,b, 1969; Kowalewski 1969), we included in our present experiment a study of the action of an anabolic steroid on the excretions of hydroxyproline in rats with healing thermal injury of skin.

**METHODS**

**Urine collection**

Female Wistar rats, were housed individually in metabolic cages and fed *ad libitum* a Purina Laboratory diet, containing 3.8 g of total nitrogen in 100 g of dry weight. Food and drinking water consumption in these animals was measured every second day. A urine specimen of each animal was collected, under 2 ml toluene, for 24 h and stored at 0°C for not more than 48 h prior to processing. Collections of urine were done during the period of healing of burns.

**Thermal injury**

Dermal burns were produced under ether anaesthesia, following our previously reported method (Kowalewski & Yong 1969). A 100 g stainless steel scale weight (a cylinder with a 2.5 cm diameter) was kept in boiling distilled water for five minutes. Then it was placed, with the full weight of 100 g against the carefully shaven skin of the rat's back, in the centre of the body. On each side of the spine, two such burns were produced. The source of heat was applied twice at the same place, each time for 60 seconds, using a freshly heated cylinder. Several heated cylinders were available. It was assumed that the base of the cylinder produced a constant caloric output at a given time, so that all animals were burned identically. It was found in preliminary experiments, that in rats with body weight of 170 g, such burns damaged about 12% of the skin surface. Histological lesions resulting from burns produced with this method were
described recently (Kowalewski & Yong 1969). We also reported on systemic and local changes in dermal collagen in rats burned by this procedure (Kowalewski & Yong 1969).

**Treatment**

The following two groups of burned rats were studied: 1) Controls, having no treatment; 2) Treated with an anabolic steroid. Treatment began immediately after injury (0 time). A depot preparation of 17β-hydroxy-17α-methylandrostan [3,2-c] pyrazole (Winstrol®, Winthrop Co.) was injected intramuscularly, 15 mg/kg body weight on days 0, 5, 10, 15 and 20 following burns. In both groups of rats the experiment lasted 30 days.

**Biochemistry**

Hydroxyproline was estimated following the procedure described by Kivirikko et al. (1967) and expressed in μg/100 g of animal’s body weight, in 24 h urine.

**RESULTS**

**Body weight, food and water consumption**

Initial and final body weights were 197.4 ± 3.3 and 238.0 ± 21.3 in controls; they were 196.2 ± 2.3 and 238.6 ± 23.7 in hormone treated rats. After thermal injury, ponderal growth was delayed between days 12 and 24 in controls, but not in treated animals. Food and water consumption did not change significantly during the experiment. It was, however, significantly higher ($P<0.01$) in hormone treated rats. Average 24 h food consumption was 7.4 ± 1.7 g in controls and 9.7 ± 0.7 g in hormone treated rats. Respective average 24 h water intakes in these groups were 7.5 ± 1.5 ml and 9.6 ± 0.7 ml.

**Hydroxyproline in urine**

In this experiment 420 24-h samples of urine were studied biochemically. Effect of thermal injury and hormonal treatment on the excretion of hydroxyproline is statistically analyzed in Table 1 and well visualized in Fig. 1. In Table 1, weekly collections of urine prior to injury and during the post-injury healing period of three weeks are added together for the statistical analysis. It may be noted from Table 1 that progressive significant increase of hydroxyproline excretion occurred in rats following injury and was maximal at third week after burns. During this week, both groups of rats differed significantly. The effect of treatment was evident only during the third week after injury.

**DISCUSSION**

Effects of thermal injury on dermal hydroxyproline and histological changes in healing skin were described recently (Kowalewski & Yong 1969). In this study...
Table 1.
Effect of thermal injury and treatment on the excretion of urinary hydroxyproline in rats. Weekly collections of urine prior to injury and during three weeks following injury were added for the statistical analysis. Number of 24 h urine samples given in brackets. Averages and sd.

<table>
<thead>
<tr>
<th>Group</th>
<th>Period of Collection</th>
<th>Urinary hydroxyproline, µg/24 h/100 g body weight</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>Treated with anabolic steroid</td>
</tr>
<tr>
<td>G. 1</td>
<td>Prior to injury days 0–7</td>
<td>232.8 ± 51.7 (70)</td>
<td>237.6 ± 55.3 (70)</td>
</tr>
<tr>
<td>G. 2</td>
<td>First week after injury days 8–14</td>
<td>266.6 ± 49.9 (56)</td>
<td>259.9 ± 44.3 (56)</td>
</tr>
<tr>
<td>G. 3</td>
<td>Second week after injury days 15–21</td>
<td>293.4 ± 54.7 (42)</td>
<td>295.3 ± 48.6 (42)</td>
</tr>
<tr>
<td>G. 4</td>
<td>Third week after injury days 22–28</td>
<td>451.2 ± 96.3 (42)</td>
<td>508.0 ± 97.3 (42)</td>
</tr>
</tbody>
</table>

P - < 0.05 G. 1 versus G. 2 and 3  
< 0.01 G. 1 versus G. 4  
< 0.01 G. 2 versus G. 3 and 4  
< 0.01 G. 3 versus G. 4  
< 0.01 G. 1 versus G. 3 and 4  
< 0.01 G. 2 versus G. 3 and 4  
< 0.01 G. 3 versus G. 4

we found a significant increase in total and soluble hydroxyproline of healing dermal burns at the end of the first and second week following injury. This increase was followed by the fall of both hydroxyprolines during the third week following burning. During these first two weeks after injury, significantly higher hydroxyproline (both total and soluble) was found in the burned skin of rats treated with Winstrol, than in the burned skin of control animals (Kowalewski & Yong 1969). It is interesting to note that this previously found decrease in tissue hydroxyproline, during the third week following injury, occurs at the same time as the increase of urinary hydroxyproline found in the present study. Because both studies are comparable regarding the method of production of thermal injury, we may assume that hydroxyproline excreted in large quantities during the third week after burns has its origin in injured healing tissue.

Urinary hydroxyproline excreted during the healing of burns is a product of both synthesis and catabolism of collagen. However, these two processes can-
Urinary excretion of hydroxyproline prior to and following thermal injury of skin in rats, treated or not with an anabolic steroid. Average values, 14 rats in each group.

not be separately identified by the study of urinary hydroxyproline. It is probable that during the first two weeks following burns, a part of the excreted hydroxyproline is the result of effort of healing connective tissue to replace the collagen destroyed by burns. However, the majority of excreted hydroxyproline represents the catabolism of collagen. In healing burned skin, necrosis is still present during the first two weeks following injury, but new fibroblasts already appeared, and the formation of ground substance is on the way (Kowalewski & Yong 1969). This may signify that both new formed collagen and broken down fibres supply hydroxyproline for excretion in the urine. Thermal injury differs from other experimental and clinical wounds produced by incision, particularly because skin protein is denatured in burns. After it has been denatured, collagen becomes susceptible to various proteinases; increase of urinary hydroxy-

545

Acta endocr. 61, 3
proline after thermal injury represents essentially this proteolytic breakdown. In patients with severe burns (Klein et al. 1962; Klein & Davis 1964), the excretion of urinary hydroxyproline reached a peak about 15 days after injury and then declined. In our present study, the peak value of post-injury excretion of hydroxyproline occurred on the seventeenth day following burns and then slowly declined. There is a rather remarkable similarity in the response of collagen to burns in humans and rats.

An effect of anabolic steroid on post-burns urinary hydroxyproline was certainly notable during the third week following injury. In the previous study, when the same method of treatment was used (Kowalewski & Yong 1969), anabolic steroid produced the significant increase in dermal hydroxyproline, but this reaction was limited to the first two weeks after injury. The same early anabolic action of this steroid was observed previously in a study of the histological healing of fractured bones (Murakami & Kowalewski 1966). It appears therefore that anabolic steroid Winstrol affects the synthesis of collagen in various healing tissues, in the first weeks following injury, but probably does not alter the final biochemical and/or histological picture of healing wounds studied 3-5 weeks after injury. The differences in the excretion of hydroxyproline observed between two groups of burned rats is considered due to the anabolic action of Winstrol. The general type of reaction of dermal collagen to thermal injury, as represented by the curves of urinary excretion of hydroxyproline is, however, not altered by the hormonal therapy of burned rats under the experimental conditions described.

Urinary excretion of hydroxyproline may be a valuable test for the study of humans and animals following thermal injury of skin. The results of the present study seem to indicate that rats, as humans, do react to thermal injury by the proteolysis of denatured skin collagen: urinary hydroxyproline increase following burns reflects adequately the alteration of collagen in injured tissue. Anabolic steroids may stimulate the metabolism of collagen, following injury, and consequently influence the amount of excreted hydroxyproline.

ACKNOWLEDGMENTS

This study was supported by a grant from Winthrop Laboratories, Aurora, Ontario, Canada, and by a grant from Medical Research Fund, The University of Alberta. Mr. G. Chmura and Mrs. T. Hoogen are thanked for their technical assistance.

REFERENCES

Laitinen D.: Acta endocr. (Kbh.) Suppl. 120 (1967) 1.

Received on December 2nd, 1969.