

Urinary ^{14}C -Hydroxyproline in Thyroxine-treated Rats after the Administration of ^{14}C -Proline

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Urinary excretion of hydroxyproline is greatly increased in rats after the administration of thyroid hormones^{1,2} and in patients with hyperthyroidism,³⁻⁶ and decreased in rats with experimental hypothyroidism³ and in patients with hypothyroidism.^{6,7} Studies with ^{14}C -proline indicate that the urinary hydroxyproline in young rats is derived both from the recently synthesized soluble collagen and from the catabolism of the mature insoluble collagen fibres.^{8,9} Thus the additional hydroxyproline excreted in hyperthy-

roidism might be derived from the soluble collagen or from the insoluble collagen or from both. The finding that the content of soluble collagen in the skin of rats was decreased in hyperthyroidism suggested that the additional hydroxyproline excreted in the urine might be derived, at least partly, from the increased catabolism of soluble collagen.¹ This question was studied further in the present work with ^{14}C -proline.

The test animals were male albino Wistar rats, which at the start of the experiments were six weeks of age. Urine was collected under toluene during the periods indicated in the tables and quantitatively analysed for hydroxyproline by the method of Prockop and Udenfriend¹⁰ with slight modifications and for the specific activity of ^{14}C -hydroxyproline by the method of Prockop *et al.*¹¹ The total activities of urinary ^{14}C -hydroxyproline were calculated from these values.

In the experiment described in Table 1 A the administration of thyroxine to the

Table 1. Effect of L-thyroxine on the urinary excretion of hydroxyproline and on the specific activity and total activity of ^{14}C -hydroxyproline after the administration of ^{14}C -proline.

A. The rats received 40 μg of L-thyroxine sodium daily subcutaneously. After 8 thyroxine injections an intraperitoneal injection of 15 μC of ^{14}C -proline in 1.00 ml of physiological saline was given. 4 rats in each group.

Collection period after ^{14}C -proline	Group	Urinary hydroxyproline		
		μg during collection	specific activity cpm/ μg	total activity cpm
0-14 h (14 h)	Controls	275 (236-351)	10.4 (7.50-11.9)	2880 (1820-3830)
	Thyroxine	478 (424-542)	5.87 (4.60-6.81)	2810 (2260-3690)
48-62 h (14 h)	Controls	269 (164-326)	2.05 (1.32-2.98)	522 (379-652)
	Thyroxine	781 (543-1310)	1.66 (1.35-1.99)	1363 (827-2610)
96-120 h (24 h)	Controls	409 (283-552)	1.71 (1.07-2.35)	682 (427-931)
	Thyroxine	914 (735-1195)	1.25 (1.18-1.29)	1148 (868-1530)

B. The rats received 25 μC of ^{14}C -proline in 1.00 ml physiological saline. The daily administration of 40 μg of L-thyroxine sodium was begun 30 days later. 3 rats in each group.

29-30 days (24 h)	Controls	512 (463-585)	0.99(0.88-1.10)	501 (478-515)
	Controls *	488 (437-541)	1.03 (0.86-1.25)	500 (433-605)
39-40 days (24 h)	Controls	493 (436-537)	0.82 (0.77-0.88)	402 (384-413)
	Thyroxine	690 (588-827)	0.98 (0.88-1.04)	679 (517-860)

* This group began to receive thyroxine.

experimental rats was begun 8 days before the administration of ^{14}C -proline. The total activity of urinary ^{14}C -hydroxyproline in the treated animals was about the same as in the controls during the first 0–14 h, but more than doubled during the period 48–62 h and almost doubled during 96–120 h. In a further experiment the results were similar, except that during 0–14 h the total activity was already about 20% higher in the thyroxine-treated rats than in the controls. By contrast, the specific activities of urinary hydroxyproline were considerably lower in the thyroxine-treated rats than in the controls. Preliminary measurements of the specific activities and total activities of hydroxyproline in the soluble and insoluble collagen fractions of the skin in a similar series 8 h and 120 h after the administration of ^{14}C -proline indicate that these values were considerably lower in the thyroxine-treated rats than in the controls. The greatly increased total activities of urinary ^{14}C -hydroxyproline, in spite of the decreased specific and total activities of ^{14}C -hydroxyproline in the skin collagen fractions, suggest that the additional hydroxyproline excreted in thyroxine-treated rats was largely derived from increased catabolism of the recently synthesized collagen. The reason for the low specific activities of hydroxyproline in the urine and skin collagen fractions in the beginning of the experiment is as yet unexplained. Since the excretion of free proline in the urine was found to be slightly greater in the thyroxine-treated rats than in the controls, it seems possible that the proline pool was larger in the thyroxine-treated rats than in the controls. This would have led to a greater dilution of the injected ^{14}C -proline and thus to a lower specific activity of the incorporated ^{14}C -proline, which would at least partly explain the lower specific activity of ^{14}C -hydroxyproline in the thyroxine-treated rats.

In the experiment described in Table 1 B the administration of thyroxine was begun 30 days after the injection of ^{14}C -proline when the urinary ^{14}C -hydroxyproline was mainly derived from insoluble collagen fibres^{8,9}. After 10 thyroxine injections the total activity of urinary ^{14}C -hydroxyproline

was about 70% higher in the thyroxine-treated rats than in the controls. In addition, the specific activity of the urinary ^{14}C -hydroxyproline was possibly slightly higher in the thyroxine-treated animals. The results of this experiment suggest that the catabolism of the mature insoluble collagen fibres formed before the start of thyroxine administration was increased by the administration of thyroxine.

The results of the present communication thus suggest that the additional hydroxyproline excreted in experimental hyperthyroidism was derived both from increased catabolism of the recently synthesized collagen and from increased catabolism of the mature collagen fibres. Further work is in progress to clarify the mode of action of thyroid hormones on the metabolism of collagen.

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