Prednisone Effect on Serum Hydroxyproline Fractions* (34122)

ANDRE C. KIBRICK AND ADE T. MILHORAT
Institute for Muscle Disease, Inc., New York, New York 10021

We reported recently that administration of prednisone produced a pronounced reduction of total urinary hydroxyproline in children with muscular dystrophy between the ages of 7 and 14 years but had essentially no effect on one patient 20 years old (1). This effect of corticosteroids on urinary hydroxyproline in young but not in older animals had been reported repeatedly (2, 3). Their effect on the specific activities of ^{14}C hydroxyproline in urine and in skin collagen fractions of rats after injections of ^{14}C proline has also been studied (4). However, little has been known of the effect of corticosteroids on serum hydroxyproline. We presented a preliminary report in 1968 that prednisone administration reduced free and total diffusible serum hydroxyproline in both young and old rats and in patients with muscular dystrophy, but that there was no significant reduction in protein hydroxyproline (5). Contrary to our findings, Morsches *et al.* had reported in 1967 that there was a significant reduction in serum protein hydroxyproline in rats on administration of prednisolone (6). The present paper is a more complete presentation of our results in patients and in rats treated with prednisone.

Materials and Methods. Rats. In two experiments Sprague-Dawley male rats of varying ages were maintained on a normal commercial diet[1] and in a third on a purified collagen-free diet consisting essentially of 27% casein supplemented with a vitamin mixture.[2] Prednisone (Schering 1%) was administered in five subcutaneous injections every other day in the following amounts: 0.2 ml for 90–110 g, 0.4 ml for 200–250 g, 0.6 ml for 300–350 g, and 1.0 ml for 500–600 g rats. The night of the last injection, food was withdrawn, and the rats were decapitated the next morning and the blood collected and allowed to clot. Except for the groups of the largest animals, the serum from 2–4 rats was pooled according to the amounts obtained. Samples of 1 ml were removed for determination of protein hydroxyproline, and all of the remaining serum was again combined to form only one pool for each group of animals.

Patients. Seven male patients with the pseudohypertrophic form of muscular dystrophy (Duchenne) between the ages of 8 and 20 years were maintained on a collagen-free diet (1) of constant caloric value. Prednisone was administered orally for long periods varying from 1 to 4½ months at dosage between 10 and 20 mg/day. Then venous blood samples were withdrawn after an overnight fast for analysis of the serum.

Analyses. Protein hydroxyproline was determined by a modification (7) of the method of LeRoy *et al.* (8) by hydrolysis in 6 *N* hydrochloric acid followed by chromatography. Free and total diffusible hydroxyproline were determined at least in duplicate by extraction in 80% alcohol, distillation *in vacuo* at 40°, and then analyzed with and without hydrolysis by the method of Prockop and Udenfriend (9).

Results and Discussion. The results of the duplicate analyses for free and total diffusible hydroxyproline agreed within narrow limits for each group of rats, and those for protein hydroxyproline were also in very good agreement for each group. The average serum values in rats appear in Table I and those in patients in Table II. It is evident from the experiments in rats that age makes very little

* This investigation was supported by Grant AM 06706 from the National Institute of Arthritis and Metabolic Diseases, NIH, and by Muscular Dystrophy Associations of America, Inc.

[1] Lab-Blox, Allied Mills, Inc.

[2] Nutritional Biochemicals, Inc.

TABLE I. Serum Hydroxyproline Fractions in Normal and in Prednisone-Treated Rats.

	Control				Prednisone-treated			
		Diffusible				Diffusible		
		Total	Free	Protein		Total	Free	Protein
		Hydroxyproline				Hydroxyproline		
Weight (g)	No. of rats	(μg/ml)			No. of rats	(μg/ml)		
Expt. 1								
90–100	8	7.4	5.35	5.6	9	3.85	2.35	5.6
200–250	6	7.3	5.35	4.8	6	3.35	2.5	5.2
400–450[a]	8	2.24	1.65	5.7	8	1.45	0.96	5.7
Expt. 2								
90–100	8	7.7	6.6	7.4	8	4.9	3.4	6.9
200	4	8.0	6.5	7.8	4	4.8	3.3	7.0
300	6	4.55	2.85	6.0	6	2.6	1.65	5.5
Expt. 3								
90–100	5	7.25	4.5	6.25	12	4.45	3.3	5.9
320	4	5.7	3.2	6.0	8	3.1	1.6	5.8
575[b]	2	4.4	2.5	5.65	2	1.85	1.16	5.5

[a] Two years old.

[b] One year 3 months old.

difference in the reduction of diffusible hydroxyproline induced by administration of prednisone. In rats 15 months and 2 years old, the reduction in free and total nonprotein hydroxyproline was 48 and 47%, respectively, whereas the reduction in younger animals varied between 27 and 58% for free, and between 35 and 54% for total nonprotein hydroxyproline. On the other hand, protein hydroxyproline was unchanged. Similarly, the reduction in patient 4, aged 20, was no different from that in the younger patients and protein hydroxyproline was unchanged in all of the patients during periods in which prednisone was given.

These results are different from the effects

TABLE II. Serum Hydroxyproline Fractions in Patients with Muscular Dystrophy in Control Periods and during Prednisone Treatment.

		Control period			Prednisone period		
		Diffusible			Diffusible		
		Total	Free	Protein	Total	Free	Protein
		Hydroxyproline			Hydroxyproline		
Patient	Age (year)	(μg/ml)			(μg/ml)		
1	8	2.8	1.38	8.15	1.27	0.82	7.78
2	15	3.24	2.04	6.71	1.42	0.85	6.64
3	14½	3.22	2.17	7.15	1.68	1.07	6.87
4[a]	20	2.01	1.31	5.59	1.39	0.70	5.65
5	8	3.09	2.11	5.92	1.15	0.65	5.95
6	8½	3.85	2.59	6.26	1.68	0.97	6.24
7	11	3.59	2.78	—	1.30	0.75	—

[a] Prednisone, 20 mg daily for 2 months.

observed on the excretion of urinary hydroxyproline. It is well known that older rats show a much smaller decrease in total urinary hydroxyproline after administration of corticosteroids than do younger animals. The considerable decrease in diffusible serum hydroxyproline after prednsone treatment in patient 4 is of interest since this is the same period (1) during which the urinary excretion of hydroxyproline of this patient was essentially unchanged. Failure to obtain differences in the concentration of serum protein hydroxyproline between periods of prednisone administration and of control shows that the corticosteroid does not affect the formation and degradation of this protein. However, the considerable reduction of diffusible hydroxyproline in older rats and in the patient aged 20 cannot be readily explained.

The data presently available suggest that the nonprotein hydroxyproline peptides of serum, containing very little prolylhydroxyproline (10), appear as urinary peptides, which are chiefly prolylhydroxyproline (11). Free hydroxyproline in serum may be disregarded for our purposes since there is little free hydroxyproline in urine. In both young animals and young patients the order of reduction in hydroxyproline peptides of serum and urine after prednisone administration usually is about the same. The mechanism which governs the formation of urinary peptides from the serum peptides is essentially not affected by the corticosteroid during the early life periods. On the other hand, in older animals this mechanism is apparently affected by prednisone. The hydroxyproline systems in serum and urine are being studied further to learn how prednisone administration differs in the two age groups.

Summary. Male rats in 3–4 age groups to a maximum of 2 years old were given five subcutaneous injections of 0.2 ml/100 g of 1% prednisone on alternate days and then fasted overnight and bled by decapitation. Seven patients with muscular dystrophy from 8–20 years old were given oral prednisone, 10–20 mg/day, for 1–4½ months and then fasted overnight before taking venous blood samples. Values of free and total diffusible serum hydroxyproline were reduced by about 50% from control values in all groups of 100–600-g rats. They were similarly reduced in all of the patients from values during control periods. Serum protein hydroxyproline was unchanged in both the rats and patients. Failure to obtain differences between old and young animals and patients in diffusible hydroxyproline is discussed.

1. Kibrick, A. C., Power, H. L., Sevendal, E., and Milhorat, A. T., J. Clin. Endocrinol. Metab. **28**, 1113 (1968).

2. Smith, Q. T. and Allison, D. J., Endocrinology **77**, 785 (1965).

3. Kivirikko, K. I. and Laitinen, O., Acta Physiol. Scand. **64**, 356 (1965).

4. Kivirikko, K. I., Laitinen, O., Aer, J., and Halme, J., Biochem. Pharmacol. **14**, 1445 (1965).

5. Kibrick, A. C., Tuohy, J., Sevendal, E., and Milhorat, A. T., Federation Proc. **27**, 763 (1968).

6. Morsches, B., Holzmann, H., and Schlaudecker, A., Arztiche Forsch. **21**, 421 (1967).

7. Kibrick, A. C., Kitagawa, G., Sevendal, E., and Milhorat, A. T., Enzymol. Biol. Clin. **6**, 171 (1966).

8. LeRoy, E. C., Kaplan, A., Udenfriend, S., and Sjoerdsma, A., J. Biol. Chem. **239**, 3350 (1964).

9. Prockop, D. J. and Udenfriend, S., Anal. Biochem. **1**, 228 (1960).

10. Kibrick, A. C., Kitagawa, G., Maskaleris, M. L., Gaines, R., Jr., and Milhorat, A. T., Proc. Soc. Exptl. Biol. Med. **119**, 622 (1965).

11. Meilman, E., Urivetsky, M. M., and Rapoport, C. M., J. Clin. Invest. **42**, 40 (1963).

Received March 3, 1969, P.S.E.B.M., 1969, Vol. 131.