Urinary Hydroxyproline Excretion in Marfan's Syndrome as Compared with Age Matched Controls.* (29412)

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Sjoerdsma, Davidson, Udenfriend and Mitoma(1) described increased urinary hydroxyproline excretion in 7 out of 10 cases of Marfan's syndrome between the ages of 17-30 years. Since then Prockop and Sjoerdsma(2) and Meilman and Urivetsky(3) have reported elevated hydroxyproline excretion in 4 more adult patients with Marfan's syndrome. Although these investigators(1-3) presented data concerning hydroxyproline excretion in normals and in miscellaneous disease states they did not specifically compare hydroxyproline excretion in Marfan's syndrome with age-matched controls. Since it has been shown that 24 hour urinary excretions of hydroxyproline vary markedly with age(4) any interpretation of hydroxyproline excretion in disease states must take this factor into account.

In this paper is presented the 24 hour urinary hydroxyproline excretion in 17 patients with Marfan's syndrome between the ages of 11 through 38 years as compared with 56 age-matched controls. This represents the largest investigation of hydroxyproline excretion in patients with Marfan's syndrome and the only such study to include Marfan's patients younger than 17 years of age.

Experimental. Each of the subjects with Marfan's syndrome had at least 3 of the following 4 criteria, as suggested by McKusick(5): a family history of the disease; luxation or subluxation of the lens; a sinus of Valsalva aneurysm with aortic insufficiency; and dolichostenomelia and redundant ligaments and joint capsules. None of the Marfan's patients in this study were of the forme fruste, or incomplete, variety. The 56 controls consisted of part of the population described elsewhere(4).

All subjects were carefully instructed and understood the importance of obtaining an accurate 24-hour urine volume. All collections were carefully supervised and if there was any doubt as to the validity of a specimen it was discarded and another was obtained. With the exception of multivitamins, none of the participants was on continuous medication nor did any have medication 48 hours prior to and during the collection period. None had an excessive intake of substances such as gelatin, bar candy, or ice cream which are known to give rise to a transient elevation in hydroxyproline excretion when taken in abnormally large quantities. Except for the presence of the stigmata of Marfan's syndrome in the experimental group, there was no other evidence of acute or chronic disease by history or physical examination.

Urines were collected under 30 ml of toluene and were analyzed immediately or stored at −10°C until analyses could be performed. Each urine was filtered and a well mixed 0.1 to 0.5 ml aliquot containing 1 to 150 μg of hydroxyproline was hydrolyzed in 6 N HCl in sealed tubes at 124°C for 3 hours. After hydrolysis, the samples were analyzed for hydroxyproline using the colorimetric method of Prockop and Udenfriend(6) with the following terminal modification as described by Woessner(7): To stabilize the final chromophore, the tubes were placed in a 60°C water bath for 20 minutes, then cooled in tap water for 5 minutes. The color that developed was read at 560 mμ on a Bausch and Lomb spectrophotometer. Values given represent total hydroxyproline which is taken to be equivalent to peptide-bound hydroxyproline.

Results. Table I presents the 24-hour hydroxyproline excretion in 17 patients with Marfan's syndrome as compared with 56 age-matched controls. As the authors have previously shown(4), it is evident that hydroxy-

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TABLE I. Urinary Hydroxyproline Excretion in Marfan's Syndrome as Compared with Age Matched Controls.

	Marfan's syndrome		Controls		
Age	No.	Hydroxy-proline (mg/24 hr)	No.	Hydroxy-proline (mg/24 hr)	
				Range	Mean
11	1	260	3	106-242	170
12	1	327	3	172-306	218
13	1	275*	2	233-348	290
14	1	512*	18	155-696	353
15	3	102 113 209	12	109-684	385
17	2	171 238	2	100-124	112
18	3	85 109* 239	2	56-125	91
23	1	131*	4	21-61	34
25	1	194	3	37-65	53
33-38	3	122 136 416	5†	13-29	23

* Patients courtesy of Dr. Victor A. McKusick, Johns Hopkins Hospital, Baltimore, Md.
† Ages 33-49.

proline excretion varies markedly with age. The 11- to 12-year-old Marfan's patients excreted greater amounts than the controls, while those 13, 14, and 15 years old had hydroxyproline excretions within normal limits. The two 17-year-old and one of the three 18-year-old Marfan's patients excreted more hydroxyproline than the respective controls. For the 13- to 18-year-old group, the variation is too great for significant difference to be seen. All 5 patients with Marfan's syndrome who were 23 through 33 years of age excreted considerably greater amounts of hydroxyproline than normal adults. Thus, pre-adolescent and adult Marfan's patients excreted greater amounts of hydroxyproline than age-matched controls, while 7 out of 10 adolescent patients had hydroxyproline levels within control limits.

Discussion. The work of Stetten(8), Wolf and Berger(9), Green and Lowther(10), Hausmann and Neuman(11), Lindstedt and Prockop(12), Prockop and Sjoerdsma(2), Prockop, Peterkofsky and Udenfriend(13), Peterkofsky and Prockop(14) and Prockop, Kaplan and Udenfriend(15), shows that endogenous and exogenous hydroxyproline do not directly participate in collagen formation. Hydroxyproline appears in the collagen molecule probably as a result of hydroxylation of collagen or pre-collagen proline *in situ*. Collagen degradation apparently yields the peptides containing hydroxyproline which appear in the blood and then are largely excreted in the urine. The amount excreted probably reflects mainly collagen catabolism.

It is of interest that the structures typically affected in Marfan's syndrome (the sclera of the eye, the fibrous root of the aorta, and ligaments, tendons, joint capsules and bones) have a high collagen composition. This together with the increased hydroxyproline excretion in some patients with Marfan's syndrome would tend to implicate the collagen fibril and/or the ground substances as the site of the basic defect in this disease.

Summary. Hydroxyproline excretion was studied in 17 patients with classical Marfan's syndrome from 11 to 33 years of age and compared with age-matched controls. Pre-adolescent and adult Marfan's consistently excreted greater amounts of hydroxyproline than controls. For the 13- to 18-year-old group, the variation was too great to show definite differences.

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Some Effects of Cations and of Water Absorption on Intestinal Hexose, Glycine and Cation Absorption.* (29413)

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Glucose absorption *in vivo* decreased upon the replacement of sodium in the rats' intestine lumen perfusate(1,2). However, various sodium and non-sodium electrolytes at given osmolar concentrations affected differently the intestinal absorption rate for water in the dog(3). Accordingly, the present experiments were designed to test whether hexose or glycine absorption in the dog is influenced by the chemical identity of the accompanying perfusate electrolyte, and whether such influences are related to concomitant changes in water absorption. Additional experiments test whether sodium, potassium and ammonium ions alter the absorption of one another, and whether changes in cation absorption rate are related to changes in net water absorption.

Methods. This technique has been described(3-5). Four dogs with jejunal and 5 dogs with ileal Thiry-Vella fistulas (approx. 20 cm long) were used. Test solutions were perfused through the loops at the rate of about 2 ml/min for one hour in fasted, non-anesthetized dogs. Each dog was tested 2 or 3 times per week. The volume and solute concentrations of solutions were measured before and after loop perfusion. After correction for fluid and solute remaining in the perfused loop, absorption or enterosorption of solute and water was considered to be that amount which disappeared from, or was added to, the perfusate. Hexoses(6) and sodium and potassium (flame photometry) were measured. Ammonium was estimated by microkjeldahl determination on test solutions and on deproteinized, non-digested intestinal effluents; traces of "ammonium N" enterosorbed by dogs receiving ammonium-free perfusates were disregarded. Glycine was estimated (microkjeldahl) on deproteinized, digested effluents, corrected for each dog by that amount of "amino acid N" enterosorbed into N-free perfusates(3,4).

In the first series, changes in water absorption rates were produced by varying the molar concentration of sodium or non-sodium salts in the perfusates while hexose or glycine was approximately 14 mMolar. Absorption rates of the non-electrolytes are reported. In the second series, varying concentrations of NaCl and KCl, NaCl and NH_4Cl, or NH_4Cl and KCl were perfused in solutions made up to approximately 310 mOsmolar by addition of glucose. Net cation sorption rates are reported. The sequence of the various mixtures was randomized in both series.

Results. Table I summarized hexose, glycine and fluid absorption (—values) or enterosorption (+values) rates when sodium, potassium or ammonium salts were present. These salts were chosen because of their differing effects on water sorption in the dog (3). Glycine was not tested with NH_4Cl or K_2SO_4. The data show that the absorption rates for all substances from jejunal and ileal loops were reduced when KCl was substituted for NaCl and when K_2SO_4 was substituted for Na_2SO_4. The substitution of NH_4Cl or 210 mMolar NaCl for 155 mMolar NaCl also reduced hexose and glycine absorption rates in most instances. However, inspection of concomitant hexose or glycine and water sorption data in Table I suggests a positive correlation between non-electrolyte and water

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