Urinary Hydroxyproline Excretion in Normal Children and Adolescents.* (28837)

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Since, in man, hydroxyproline is found only in collagen in significant amounts(1,2), urinary hydroxyproline excretion has been used as an index of collagen metabolism(3-7).

Prockop and Sjoerdsma(5), Ziff and coworkers(3), and Jasin *et al*(6) have shown that hydroxyproline excretion is greater in children than in adults but they did not select a narrow enough class interval to demonstrate close age specific peaks.

Using a class interval of one year, the results of a study of hydroxyproline excretion in 80 normal subjects between the ages of 5-49 is presented here. Since Prockop and Sjoerdsma(5) and Mitoma *et al*(4) found that hydroxyproline excretion does not vary significantly on low hydroxyproline or unrestricted diets, all participants in this study were on an *ad lib* diet.

Experimental. Twenty-four hour urinary hydroxyproline was measured in 70 males and 10 females between the ages of 5 through 49 of whom 11 were hospitalized and 69 were not hospitalized. All subjects and parents were carefully instructed and understood the importance of obtaining an accurate 24-hour urine volume.

The hospitalized group consisted of patients admitted for elective herniorrhaphies at St. Luke's Hospital and Metropolitan Hospital in New York City. Except for the presence of a non-incarcerated, unilateral, indirect inguinal hernia, these patients were normal in all other respects. All urines were collected prior to surgery, and collections were closely supervised by nursing personnel and authors.

The non-hospitalized group consisted of junior and senior high school students and their families from New Hyde Park, N. Y., and medical students from New York Medical College. The children from New Hyde Park were students in an accelerated science program who volunteered, as did the medical students, to participate in this study. These urines, and those of the medical students, were collected at home, and when necessary, were under the supervision of instructed parents. Upon collection of the specimen, paritcipants and/or their parents were questioned about the accuracy of the collection. If there was any doubt as to the validity of a specimen, it was discarded. In 3 instances 2 or more 24-hour urines were obtained and daily variations in urine volume and hydroxyproline excretion were compared.

With the exception of mulitvitamins, none of the subjects was on continuous medication nor did anyone 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 a transient elevation in hydroxyproline excretion when taken in abnormally large quantities(3,5). Except for the presence of unilateral, non-incarcerated, indirect inguinal hernias in the hospitalized 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 were stored at —10°C until analyses could be performed. Each urine was filtered and a well-mixed 0.1 ml aliquot containing 0.5 to 100 μg of hydroxyproline was hydrolyzed in 6N 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(8) with the following terminal modification as described by Woessner(9): To stabilize the final chromophore, the tubes were placed in a 60°C water bath for 20 minutes, then

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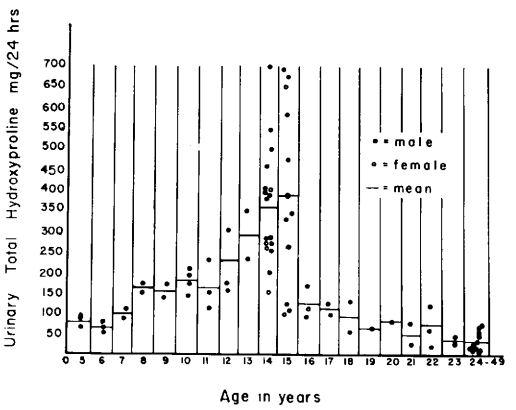

FIG. 1. Twenty-four hour urinary total hydroxyproline excretion by 80 normal subjects from 5 to 49 years of age on an *ad lib.* diet.

cooled in tap water for 5 minutes. The color that developed was read at 560 mμ on a Bausch and Lomb spectrophotometer. Values given in the text represent total hydroxyproline.

Results and discussion. Fig. 1 shows that normal values for excretion of hydroxyproline varied markedly with age. Hydroxyproline became elevated just prior to puberty then increased rapidly during puberty and early adolescence until a peak was reached during the 14th and 15th years. This high level then declined rapidly until adult levels were attained in the late teens and early twenties. Stuart(10) describes 5 periods of growth delineated as follows: slow growth, from 2 to 6 years; steady growth, from 6 to 12 years; accelerating growth, from 12 to 14 years; maximum growth, from 14 to 16 years; and decelerating growth from 16 to 20 years. When these periods are imposed upon Fig. 1, it becomes evident that hydroxyproline excretion correlates well with growth thus supporting the results of Jasin, Fink, Wise and Ziff(6), who suggested that there is an increase in the more rapidly metabolized soluble forms of collagen during periods of growth.

Although the sample is inadequate for definitive interpretation, adolescent females tended to excrete lower levels of hydroxyproline than males of the same age. This difference, however, may be related to the earlier growth of females than males(10) and to the fact that all but one of the females was in the 14 to 16 year group.

Table I shows excretion of urinary total hydroxyproline on consecutive and random days by an 8-year-old male on an *ad lib* diet. The hydroxyproline was constant, ranging from 100 to 116 mg per 24 hours, while urinary volume varied from 685 to 1140 ml; thus, urinary hydroxyproline excretion was not related to rate of urine formation. Similar results were obtained for a 14-year-old female who excreted 271 mg and 260 mg of hydroxyproline on consecutive days with urine volumes of 1110 ml and 980 ml, and for a 15-year-old male who excreted 669 mg and 651 mg of hydroxyproline with urine volumes of 1590 ml and 1200 ml. These results are consistent with those of Dull and Henneman(7) who used a normal adult maintained on a constant low hydroxyproline diet, as well as those of Prockop and Sjoerdsma(5) who showed that hydroxyproline excretion is constant whether an individual is hydrated or dehydrated, or on an *ad lib,* low hydroxyproline, or low protein diet.

TABLE I. Urinary 24-Hour Hydroxyproline Excretion by an 8-Year-Old Male on an *Ad Lib* Diet.

Date	7/2	7/3	7/10	7/13	7/24	7/26
Urine vol (ml)	853	1140	715	732	685	930
Total hydroxyproline (mg)	100	108	114	115	110	116

Summary. Twenty-four hour urinary total hydroxyproline was measured in 70 normal males and 10 normal females between the ages of 5 through 49. Normal values for excretion of hydroxyproline were found to vary markedly with age and correlated well with periods of growth.

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A New Class of Diuretic-Saluretic Agents, The α,β-Unsaturated Ketone Derivatives of Aryloxyacetic Acids. (28838)

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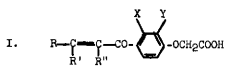
The chemistry of a new class of diuretic agents has recently been described by Schultz *et al*(1). We have studied these compounds in the dog and find that their activity differs in several ways from that of the thiazides, the organomercurials or other previously described saluretic or diuretic agents with which we have worked. In this paper are reported briefly the biological properties that are characteristic of these α,β-unsaturated ketone derivatives of aryloxyacetic acids (I). Their biological activity is illustrated by data from experiments in which the following specific examples were used:

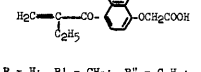


I.

II. R,R',Y = H; R" = C_2H_5; X = Cl — 3-Chloro-4-(2-methylenebutyryl)-phenoxyacetic acid

III. R,R' = H; R" = C_2H_5; X,Y = Cl — 2,3-Dichloro-4-(2-methylenebutyryl)-phenoxyacetic acid

IV. R,R' = H; R" = C_2H_5; X,Y = CH_3 — 2,3-Dimethyl-4-(2-methylenebutyryl)-phenoxyacetic acid

V.  — 4-(2-Methylenebutyryl)-naphthyloxy-acetic acid

VI. R = H; R' = CH_3; R" = C_2H_5; X,Y = Cl — 2,3-Dichloro-4-(2-ethylidenebutyryl)-phenoxyacetic acid

Methods. Trained unanesthetized fasted female dogs were used in the experiments. In the renal clearance studies, glomerular filtration rate (GFR) was estimated using creatinine, injected subcutaneously. A pH 7.4 phosphate buffer containing 4% mannitol was administered intravenously at 3.0 ml/min to provide a urine flow of 1.0-4.0 ml/min. The dogs received no other salt supplementation prior to or during these experiments. Replicate clearance periods were of 7.5-15 minutes' duration, the bladder being emptied through an indwelling catheter with washout. Each experiment described was substantiated by one or more replicate experiments in different dogs. In addition, the results cited for one compound are typical of those obtained with one or more of the other compounds I-VI, these data in turn being substantiated for each compound by 2 or more experiments in different dogs. The analytical methods were similar to those previously described (2,3), except that sodium and potassium were determined using the Autoanalyzer flame photometer.

Results and discussion. Acutely, the compounds produced a prompt and dramatic saluretic-diuretic effect when given intravenously or orally to dogs. Thus, a single intravenous dose of 1.0 mg/kg of III caused a rapid increase in excretion of sodium, chloride and water. The maximal natriuretic effect was much greater than was obtained with the same dose of hydrochlorothiazide (Fig. 1). The peak effect diminished rapidly, but, even at the end of an hour, the rate of sodium excretion exceeded the highest rate obtained with the thiazide. A total of 63.3 meq of sodium was excreted in the first hour. Although potassium excretion was also enhanced by III, the increase was less marked