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ISOTACHOPHORESIS FOR THE DETERMINATION OF OXALATE IN UNPROCESSED URINE

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SUMMARY

The principle of isotachophoresis has been used to develop a simple, specific and sensitive analytical procedure for the determination of oxalate in unprocessed urine. Analytical conditions were optimized. The accuracy and precision of the method were estimated. The specificity was checked with oxalate decarboxylase. Separation of oxalate from a number of organic acids was achieved. The influence of factors such as storage, calcium concentration, pH or ionic strength was examined.

The 24-h urine excretion rates for healthy children, healthy adults and for patients with idiopathic stone formation were established. Lower absolute excretion rates were found in children and females. Urinary oxalate/creatinine ratios were higher in children than in adults. The mean oxalate excretion in 24-h urines of adult healthy individuals was 413 ± 150 μmol per 24 h per 1.73 m^2 (range 195—732). The mean oxalate/creatinine ratio was 0.033 ± 0.011 (range 0.018—0.065).

INTRODUCTION

In clinical chemistry there is a need for a simple, rapid and specific method for the determination of oxalate in urine. None of the currently existing methods meets all these requirements and none of the existing methods allows the determination of oxalate in unprocessed urine.

Previously, Everaerts et al. [1] demonstrated that carboxylic acids can be quantitated in aqueous solutions by isotachophoresis. In a preliminary communication [2] it has been demonstrated that the isotachophoretic measurement of oxalate in aqueous samples and urines is possible without pretreatment procedures.

It is the aim of the present investigation to give a complete and detailed

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description of this new method and to present some results of its application to human urines in health and disease.

PATIENTS AND METHODS

Experimental

The isotachophoretic separations and determinations were performed in an LKB 2127 Tachophor (LKB, Bromma, Sweden) equipped with the 23-cm Teflon capillary (I.D. 0.5 mm).

The starting voltage was 2 kV with the final voltage varying between 4 and 8 kV at 150 μA and 12°C. UV absorption at 254 nm was used for quantitation. Reliable identification of oxalate by the specific step height of the thermal signal was possible when the injected oxalate content exceeded 2 nmol. The time for analysis depended on the electrolyte content of the sample and varied between 20 and 60 min. The chemicals used were generally commercially available and of analytical grade. The water used was twice-distilled. Oxalate decarboxylase (EC 4.1.1.2) was purchased from Sigma (Munich, G.F.R.; No. 0-3500).

Electrolytes

Leading electrolyte: HCl Suprapur (E. Merck, Darmstadt, G.F.R.) 5×10^{-3} *M*, NaCl 1×10^{-3} *M*, 0.4% HPMC (Methocel 90 HG, 15,000 cps; Dow Chemical, Midland, MI, U.S.A.). No adjustment of pH (2.2) was carried out.

Terminating electrolyte: acetic acid 5×10^{-3} *M* (p.a. grade; Merck).

The electrolyte solutions were replaced after each run. In addition, the purification procedure for the terminating electrolyte solution was performed as proposed by Everaerts et al. [1].

Injection

The samples were injected using 10-μl Hamilton microsyringes (for some experiments Model 700 with stainless-steel cannula and plunger, and for routine purposes Model AA 701 SN equipped with a platinum needle and tungsten plunger, were used). The syringes had been siliconized twice before use. The samples were injected through the septumless syringe injector, as described by Fredriksson [3], into the leading electrolyte, the injection volume being adjusted to give at least 1 nmol of oxalate. The injection volume varied between 2 and 10 μl (leading electrolyte: 10×10^{-3} *M* NaCl).

Evaluation

The quantity of oxalate was determined by measuring the zone length with a graticule or a ruler. The usual chart speed was 0.5—2 mm/sec. For the reasons given below, calculation of the oxalate content of the samples was performed using the combined zone lengths of both the UV-absorbing and UV-non-absorbing zone of oxalate present in each sample examined (Fig. 1). Though oxalate shows a weak UV absorption at 254 nm, for better discrimination the term "UV-non-absorbing zone" is used in the following.

Treatment with oxalate decarboxylase

The effect of oxalate decarboxylase treatment on urinary or aqueous solutions of oxalate was tested at a final enzyme concentration of 0.2 U/ml at pH 3 and 37°C. The pH was kept constant by using either the citrate buffer as proposed by Hallson and Rose [4], or a 20 mmol phthalate—HCl buffer. Because of its pronounced UV absorption phthalic acid can be easily detected by isotachophoresis. The incubation time varied between 2 min and 16 h.

Urine samples

Urines were collected under outpatient conditions without dietary restrictions from 17 healthy children, 27 healthy adults, 18 adult idiopathic renal stone formers and two individuals with congenital hyperoxaluria. Urines were collected in plastic bottles kept at 4°C during the collection period. Concentrated $ZnCl_2$ (final concentration 10 mmol/l) was then added and urinary oxalate measured within 12 h after collection of the above urines.

RESULTS

Measurement of oxalate in aqueous solutions and urine samples

Aqueous solutions of oxalate were tested with concentrations in the range 0.1—10 mmol/l. The injected volume was adjusted so that the amount injected was 1—10 nmol of oxalate. As shown for urine in Fig. 1, a small UV-absorbing zone was recorded which was followed by a UV-non-absorbing zone. Both zones had high isotachophoretic mobilities. The lengths of the UV-absorbing and the UV-non-absorbing zones increased in parallel with increasing oxalate concentrations. In all aqueous or urinary solutions, these two zones were the first zones that could be detected by the UV detector.

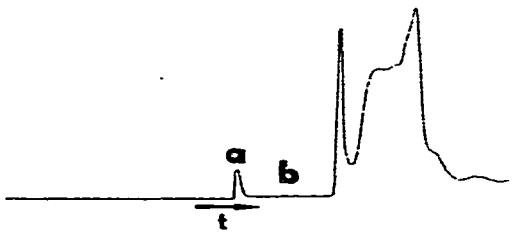

Fig. 1. Isotachopherogram (UV detection) of a 24-h urine sample. Only the initial zones are shown. Injected volume 10 μl; calculated oxalate concentration 470 μmol/l; injection with Hamilton microsyringe Model 700. (a) UV-absorbing zone of the assumed ferrioxalate complex; (b) UV-non-absorbing zone of oxalate.

When Model 700 Hamilton microsyringes (steel needle, steel plunger) were used, considerable problems were encountered, which could be avoided when Model AA 701 SN (see below) was used. The relative contribution of the UV-absorbing zone increased from 5—10% to 10—30% when Model 700 syringes had been in use for more than one week. This observation suggested that the first UV-absorbing zone might represent oxalate in complex form, probably due to interaction of oxalate with cations released from the surfaces of the syringe. Evidence for this assumption was provided by treating aqueous solu-

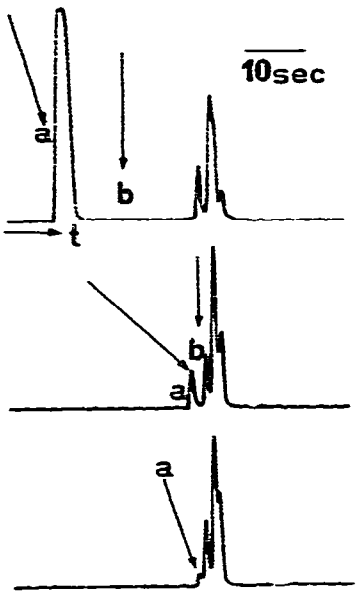

Fig. 2. Isotachophoretic monitoring of enzymatic decarboxylation of oxalate in aqueous solution by oxalate decarboxylase (EC 4.1.1.2). Conditions: oxalate 1 mmol/l, oxalate decarboxylase 0.2 U/ml, pH 3, citrate buffer after the method of Hallson and Rose [4]. The samples were injected with Hamilton microsyringe Model 700. Oxalate was measured isotachophoretically immediately, after 10 min and after 30 min of addition of the enzyme. After 30 min only a small shoulder of the initial UV-positive zone can be detected. For details see text. (a) UV-absorbing zone of oxalate (ferrioxalate complex); (b) UV-non-absorbing zone of oxalate.

tions of oxalate with oxalate decarboxylase, which decarboxylates oxalic acid specifically; under the influence of the enzyme both zones were markedly diminished and disappeared completely after 30 min (Fig. 2). It was further observed that the UV-absorbing zone of oxalate could be greatly increased by adding ferric ions as chlorides [2] or nitrates [5]. As all steel surfaces in contact with air are covered by a layer of ferri oxide, the first UV-absorbing zone of oxalate is probably a ferrioxalate complex. In aqueous solutions of oxalate, Fredriksson [5] observed that the UV-absorbing zone disappeared after addition of EDTA. In urine, however, addition of EDTA (up to 50 mmol/l), EGTA (50 mmol/l), desferrioxamine (10 mmol/l) or oxaloacetate (50 mmol/l) did not significantly influence the UV-absorbing zone, though treatment with oxalate decarboxylase affected both zones in a similar manner as in aqueous solutions (Fig. 3). The difference between aqueous and urine samples remains unexplained. Addition of spermine, spermidine or putrescine, which are known to form salts or complexes with oxalate [6], did not affect the relative length of the UV-positive or UV-negative zone of oxalate.

A significant reduction in the UV-absorbing zone of oxalate in urine samples was observed when Model 701 syringes with platinum needles and tungsten plungers were used, which had been siliconized twice before use (Hamilton microsyringe Model AA 701 SN). Any possible interaction of urine with the septum and septum bleed was avoided by using the septumless injector as described by Fredriksson [3].

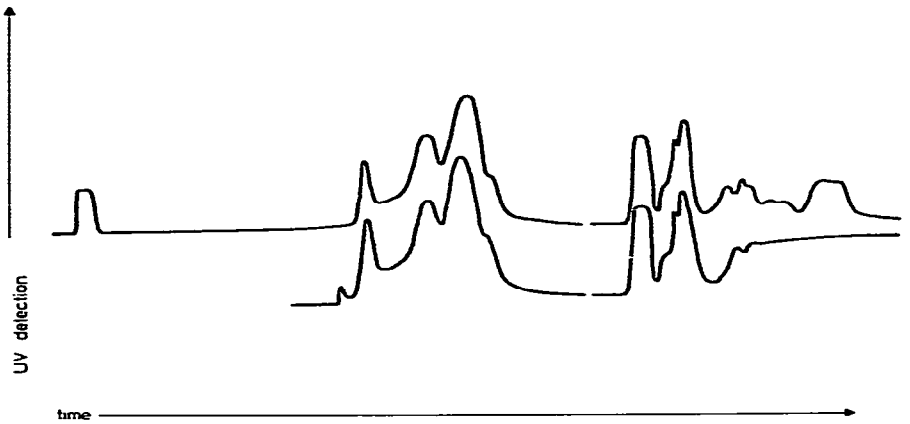

Fig. 3. Isotachophoretic run (UV detection) of a 24-h urine sample of a 11-year-old female child with congenital hyperoxaluria (oxalosis type I). The sample was analyzed by isotachophoresis both before and after treatment with oxalate decarboxylase (0.2 U/ml, incubation time 12 h, pH 3, 20 mmol phthalate—HCl buffer). Treatment with oxalate decarboxylase resulted in a significant, but not complete, disappearance of both zones of oxalate (lower curve). Injected volume 10 μl; time of analysis 40 min; calculated oxalate concentration 1150 μmol/l; injection with Hamilton microsyringe Model 700, which had been in use for two weeks.

It follows from the above experiments that both the UV-positive and the UV-negative zones contain oxalate. Consequently, for quantitation both zones have to be measured. Oxalate concentrations in unknown samples were calculated from the combined zone length of both zones. The lengths of the UV-negative and UV-positive zones were added and the oxalate concentration in the sample was calculated by comparing the sum of the zone lengths with the sum of the zone lengths of standard solutions. There was a linear relation between the zone lengths (UV-positive plus UV-negative zone) and oxalate concentration in aqueous solutions between 0.1 and 10 mmol/l (r=0.998) and in urine between 0.1 and 2 mmol/l (r=0.995).

Reproducibility

Aqueous solutions of oxalic acid or sodium oxalate (1—4 nmol injected) gave excellent reproducibility (C.V. in ten repetitive determinations was 1.2%). In 24-h urine samples, ten repetitive measurements of five samples gave a C.V. of 3.2%, when the amount of injected oxalate was adjusted to at least 1—2 nmol.

Recovery

In 24-h urines the recovery of added oxalate (1 mmol/l) varied between 70 and 100%, probably due to crystal or complex formation. While the addition of EDTA, EGTA, magnesium, citrate, or the use of citrate as terminating electrolyte were all ineffective, rates of recovery could be markedly improved by the addition of $ZnCl_2$ (final concentration 10 mmol/l). Using $ZnCl_2$ as an additive to urine, 102% of added oxalate (range 80—111) was recovered when the total amount of oxalate injected was at least 1 nmol. The effect of the addition of $ZnCl_2$ to ten different urines to which oxalate and/or calcium chloride had

TABLE I

EFFECT OF ZINC IONS ON ISOTACHOPHORETIC RECOVERY OF URINARY OXALATE IN URINES, TO WHICH OXALATE, CALCIUM, OR BOTH HAD BEEN ADDED

Full recovery of 1 mmol/l added oxalate can be achieved even in the presence of additional 10 mmol/l $CaCl_2$. Without zinc, the addition of $CaCl_2$ results in significantly lower recovery rates of added oxalate.

No.	Added to urine samples:			
	$ZnCl_2$ 10 m*M*	$ZnCl_2$ 10 m*M* Oxalate 1 m*M*	Oxalate 1 m*M* $CaCl_2$ 10 m*M*	$ZnCl_2$ 10 m*M* Oxalate 1 m*M* $CaCl_2$ 10 m*M*
1	321	1366	1151	1348
2	303	1276	910	1285
3	116	1214	1000	1160
4	411	1392	1285	1392
5	821	1803	1285	1785
6	232	1178	553	947
7	419	1482	1017	1428
8	232	1312	1089	1160
9	249	1249	1026	1249
10	276	1339	741	1267
Oxalate (μmol/l) $\bar{x}$	338	1361	1005	1302
S.D.	191	179	228	218

been added, is depicted in Table I. In the presence of $ZnCl_2$, the recovery of exogenous oxalate was still complete in the presence of high concentrations of calcium (up to 15 mmol/l). In urine samples, optimal recovery was not observed before 2 h after addition of $ZnCl_2$. Therefore, in all experiments urines were examined at least 5 h after the addition of $ZnCl_2$.

Specificity

Since organic acids other than oxalic acid are present in urine, a variety of acids with comparable mobilities [1] were examined. Specimens containing the respective organic acid and oxalic acid in aqueous solutions and/or urine samples were analyzed by isotachophoresis. No evidence of interference was found. Furthermore, the addition of derivatives of oxalic acid, i.e. parabanic acid or oxaluric acid (Fig. 4), resulted in zones that were clearly separable from oxalic acid by either the thermal signal or the UV signal. Some compounds may cause interference under strong conditions by releasing or forming oxalic acid. Oxalic acid could be detected in significant amounts after hydrolysis (120—150°C, pH 3, aqueous solution) of oxalacetic acid, parabanic acid and oxaluric acid. This is in accordance with previous observations by Hodgkinson and Zarembski [7]. The oxalic acid released under these conditions could be completely decarboxylated by oxalate decarboxylase. Oxalate decarboxylase did not attack native oxaluric acid (Fig. 5).

Influence of pH, ionic strength, and storage

Changing the pH of the urine samples from 4 to 8 did not influence the results. Changing ionic strength by increasing the sodium chloride concentra-

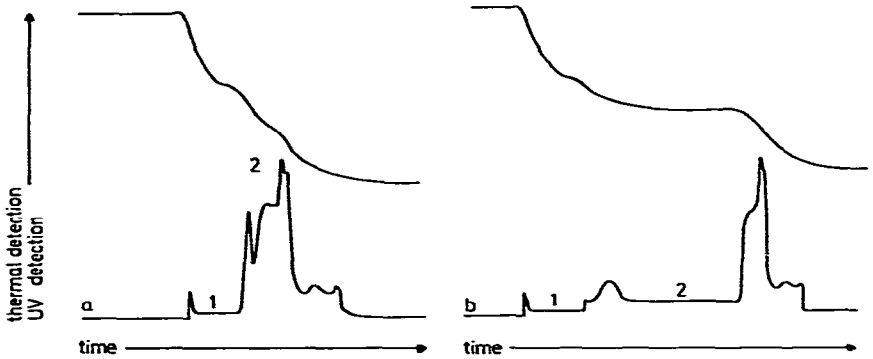

Fig. 4. Isotachopherogram (thermal detection and UV detection) of a 24-h urine sample, both before (a) and after (b) addition of oxaluric acid (1 mmol/l). Oxaluric acid has a high isotachophoretic mobility and is recorded with weak UV absorption. In higher concentrations it can be reliably separated from oxalic acid by its significantly different thermal step height. In all urine samples, oxaluric acid is separated from oxalate by yet unidentified substances, which precede oxaluric acid and show pronounced UV absorption. The traces of the thermal signal have been adapted to the UV traces for better comparison. 1 = Oxalic acid; 2 = oxaluric acid.

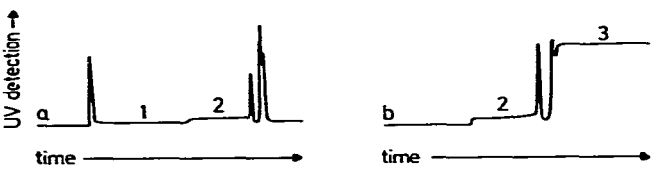

Fig. 5. The effect of oxalate decarboxylase treatment on an aqueous sample containing oxalic acid and oxaluric acid. (a) Aqueous mixture of oxalic acid and oxaluric acid (10 nmol of each substance injected). (b) Isotachophoretic record of the same sample, which has been incubated with oxalate decarboxylase in a 20 mmol phthalate—HCl buffer (pH 3). Both zones of oxalate completely disappeared from the isotachopherogram after 30 min incubation. 1 = Oxalic acid; 2 = oxaluric acid; 3 = phthalic acid.

tion in urine samples did not markedly influence recovery rates (± 5%) but increased the time of analysis considerably (up to 90 min). Significant changes of measured oxalate concentration were seen after storing. At both 4°C and −25°C, up to 20% of all urine samples showed deviations in an unpredictable way, i.e. decreases or increases of up to 20%. This has also been observed by other investigators [8].

Effect of oxalate decarboxylase treatment on isotachophoretic measurement of oxalate in aqueous and urine samples

In aqueous samples of oxalate, addition of oxalate decarboxylase (final concentration 0.2 U/ml, incubation time 2—30 min, pH 3, citrate buffer) caused complete disappearance of both the UV-positive and the UV-negative zones (Fig. 2). In contrast, in urine samples neither the UV-positive nor the UV-negative zone disappeared completely after treatment with decarboxylase for up to 16 h, although there was a parallel decrease in both zones (Fig. 3). Such a failure of oxalate decarboxylase to remove all the oxalate in urine may be related to the presence of substances in urine which are inhibitory to the enzyme.

It could be shown that both phosphate and sulfate up to 20 mmol/l were strong inhibitors of decarboxylase in aqueous solutions. While, in the absence of phosphate or sulfate, no oxalate was demonstrable after 30-min incubation under the conditions mentioned above, about 70% of the initial amount of oxalate was still present after 30 min in the presence of 20 mmol/l phosphate or sulfate. However, with both inhibitors prolonged incubation for 2.5 h still led to complete disappearance of oxalate in aqueous solutions. This was not the case in urinary solutions of oxalate, suggesting that additional inhibitory substances might be present or that other physico-chemical factors such as crystal formation or protein binding might be operative. Distinction between these possibilities for inhibition of oxalate decarboxylase in urine requires more detailed kinetic analysis.

Co-determination of other substances

A variety of substances that are important in renal stone disease (phosphate, citrate, glycolate) could be easily separated in aqueous solutions using the operational system given above. In an attempt to detect these and other acids in urine, the following substances could be detected by "spiking" (UV-positive substances) or "spacing" (UV-negative substances): oxalate, oxaluric acid, orotic acid, phosphate, citrate, glycolate, formate (Fig. 6). Quantitative co-determination of urinary citrate was possible with high accuracy and precision [9].

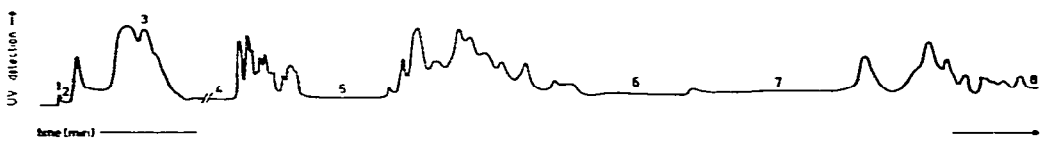

Fig. 6. Complete isotachopherogram (UV detection) of a 24-h urine sample with low oxalate content, to which glycolic acid (1 mmol/l) has been added. A number of anionic substances could be identified by "spiking" for UV-absorbing substances and "spacing" for non-UV-absorbing substances: 1 = ferrioxalate complex of oxalate; 2 = UV-non-absorbing zone of oxalate; 3 = orotate; 4 = phosphate; 5 = citrate; 6 = glycolate; 7 = formate; 8 = acetate (terminator). For quantitation of glycolate, other operational systems might be preferable, since at low concentrations of glycolate no clear-cut separation is possible from other anions either by the UV signal or by the thermal signal. Glycolate can be recognized without difficulty, however, in urine of patients with primary hyperoxaluria type I, who usually excrete high amounts of glycolate.

Urinary oxalate in healthy individuals and in renal-stone patients

As shown in Table II, urinary oxalate was lower in children than in adults. Urinary oxalate excretion was higher in adult males than in adult females [495 ± 192 (S.D.) μmol per 24 h vs. 336 ± 81 (S.D.) μmol per 24 h]. However, if oxalate excretion was corrected for urinary excretion of creatinine (thus accounting for differences in muscle mass and/or incomplete urine collection) no difference was present between male and female adults [34 ± 13 (S.D.) μmol/mmol creatinine vs. 31 ± 6 (S.D.) μmol/mmol creatinine].

The frequency distribution of the oxalate/creatinine ratio in 44 healthy individuals (age 3—48 years, weight 14—120 kg, 17 female, 27 male) is given in Fig. 7. Within the age bracket studied, i.e. if children were included, a signifi-

TABLE 2

OXALATE EXCRETION, MEASURED BY ISOTACHOPHORESIS, IN HEALTHY CHILDREN, HEALTHY ADULTS AND IDIOPATHIC STONE FORMERS

	Healthy children (6 F, 11 M)		Healthy adults (10 F, 17 M)		Idiopathic stone formers (8 F, 10 M)	
	$\bar{x}$ ± S.D.	Range	$\bar{x}$ ± S.D.	Range	$\bar{x}$ ± S.D.	Range
Age (years)	8.4 ± 3.6	3—14	28 ± 8.5	17—48	37 ± 14	18—70
Weight (kg)	28.1 ± 10.9	14—48	68.4 ± 17.5	45—120	73 ± 11.2	57—93
UV_{Ox} (μmol per 24 h)	292 ± 162	87—650	436 ± 176	190—815	471 ± 195	207—871
Oxalate per 1.73 m^2 body surface (μmol)	488 ± 203	148—801	413 ± 150	195—732	445 ± 186	190—878
Oxalate/ creatinine (μmol/ mmol)	0.066 ± 0.021	0.034—0.095	0.033 ± 0.011	0.018—0.065	0.034 ± 0.011	0.015—0.057

cant correlation could be found between UV_{Ox} on the one hand and age, weight, body surface or UV_{Cr} on the other hand (Fig. 8). However, within the groups of adult individuals, no such correlations existed. Normalization of urinary oxalate for urinary creatinine seemed to be the best approach to express urinary oxalate excretion (Fig. 8, Table II). A correlation between the urinary excretion rate of calcium and urinary excretion rate of oxalate was found, which was modest (r=0.3) in females and more marked (r=0.6) in males. The day-to-day variability of urinary excretion of oxalate was examined in five subjects on three consecutive days; the C.V. was 15%.

Urinary oxalate was also measured in two female children with congenital hyperoxaluria and recurrent stone formation (patient 1: 11 years, 3 operations,

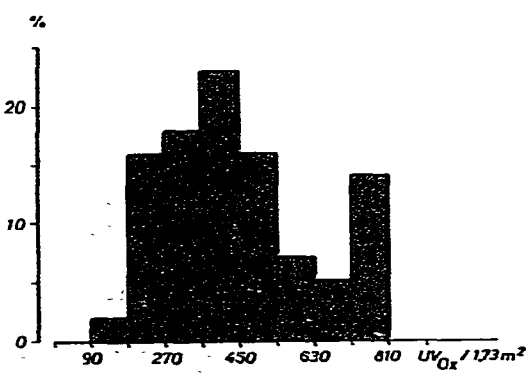

Fig. 7. Distribution of urinary oxalate/creatinine ratio in 44 healthy individuals [17 children (6 F, 11 M), 27 adults (10 F, 17 M), age 3—48 years, weight 14—120 kg].

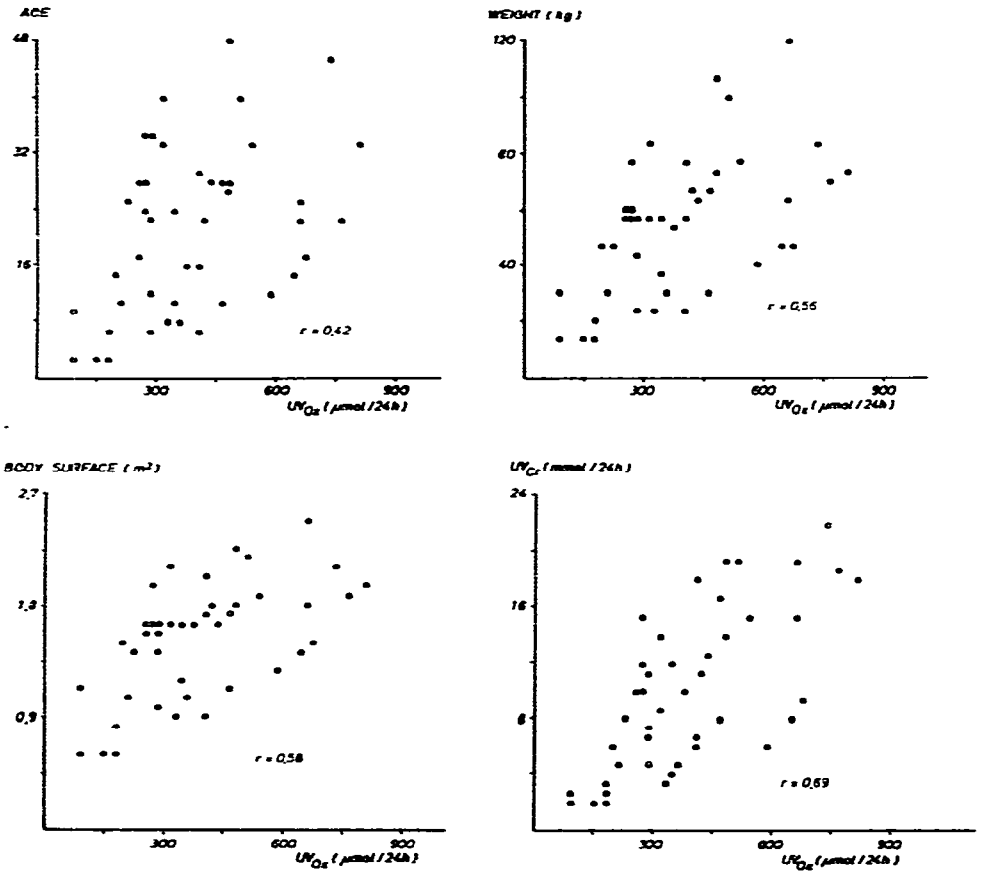

Fig. 8. Correlation of oxalate excretion rates with age, weight, body surface and urinary excretion of creatinine in 44 healthy individuals (children and adults).

GFR 80 ml/min; patient 2: 17 years, 9 operations, GFR 60 ml/min). Urinary oxalate in patient 1 was 1170 μmol per 24 h, in patient 2, 1890 μmol per 24 h. The isotachopherogram of a 24-h urine sample of patient 1 is given in Fig. 5b, before and after treatment with oxalate decarboxylase.

DISCUSSION

Isotachophoresis is based on the principle that the net mobility of each of the participating ions is constant under defined conditions. At equilibrium, therefore, the ion concentration in each unit length of a given zone is constant. Quantitation can then simply be achieved by measuring the length of each sample zone.

Oxalic acid is characterised by two dissociation constants; pK_{A^1} 1.19 and pK_{A^2} = 4.21. The low pH of the leading electrolyte solution was chosen for two reasons: first, oxalate is most soluble at low pH; second, at pH 2.2 nearly all oxalic acid exists as the monoprotonated species, $HC_2O_4^-$. The use of a pH at which oxalate is present almost exclusively as one molecular species offers

considerable advantages since, at other pH levels, determination of oxalate could be difficult because of its unique properties as a complexing agent [10, 11].

Isotachophoretic measurement of urinary oxalate has the advantage of permitting examination of urine without pretreatment such as precipitation steps, extraction steps, ion-exchange chromatography. Furthermore, the technique is rapid, simple and easy to perform. Finally, co-determination of other substances involved in lithogenesis (e.g. citrate, glycolate) can easily be carried out.

Previous investigations [1, 2, 5] demonstrated that oxalate can be measured by isotachophoresis in aqueous solutions. The measurement is complicated by the fact that a UV-positive zone, presumably consisting of Fe—oxalate complexes, precedes oxalate which is registered as a UV-negative zone. The present study demonstrates that the technique used to measure oxalate in aqueous solutions is unsatisfactory for urine samples. However, formation of the putative Fe—oxalate complex can be avoided with proper modifications (reduction of the Fe-containing surfaces by special syringes). Furthermore, it could be demonstrated that addition of zinc chloride considerably improved reproducibility and recovery of isotachophoretic measurements of oxalate in urine. The mechanism by which zinc chloride improves recovery (virtually 100%) has not been completely clarified, but it is suggestive to assume that complex formation takes place [10, 11]. With these modifications (syringes with low Fe content, addition of Zn) oxalate can be measured satisfactorily in urine samples.

Quantitation of oxalate must take into consideration the fact that oxalate is contained in both the UV-positive and the UV-negative zones, as demonstrated by the virtual disappearance of both zones after incubation with oxalate decarboxylase. It has not been strictly demonstrated that the molar concentration of oxalate in both zones is identical. However, comparison of standard curves in aqueous solution and recovery curves in urine samples demonstrate that the resulting error, if any, must be very small.

It is difficult to compare the normal range, as found in the present study, with data in the literature, since no absolute reference method exists. Furthermore, the marked dependence of urinary oxalate excretion rate on body surface, as confirmed in the present study, makes it impossible to evaluate data in the literature which are not normalized for body surface area. In sixteen adult subjects, Gibbs and Watts [12] found a range of 24—49.3 mg of anhydrous oxalic acid per 24 h per 1.73 m^2, corresponding to 266—548 μmol per 24 h per 1.73 m^2. Hodgkinson and Williams [13] reported a range of 17.2—46.8 mg of anhydrous oxalic acid per 24 h per 1.73 m^2, corresponding to 191—520 μmol per 24 h per 1.73 m^2, in 24 adults. These values are somewhat lower than those reported in the present study, but this may be a result of the small sample size in previous studies which may be particularly conducive to error because of the non-normal distribution of urinary oxalate excretion rates. With isotopic dilution methods, the problem of incomplete recovery may arise, since Gibbs and Watts [12] noted that radioactive oxalate equilibrates with some fraction of urinary oxalate which does not extract into organic solvents. Whether this fraction is identical with the oxalate still present in urine even after prolonged incubation with oxalate decarboxylase, remains conjectural. Although a number

of investigators reported differences in absolute oxalate excretion rate between males and females, the present study clearly demonstrates that urinary oxalate excretion rates, normalized for body surface, are identical in males and females. This finding is in agreement with previous studies of Gibbs and Watts [12] and Hodgkinson and Williams [13]. In contrast, urinary oxalate excretion rates, normalized for body surface area, were higher in children than in adults. This result is also in agreement with previous observations [14]. The reason for higher urinary oxalate in children is unknown, but it may be due to more effective intestinal calcium absorption in children, which is known to lead to a higher fractional intestinal absorption of oxalate [15]. Urinary oxalate, when studied under conditions of no dietary restriction, showed little day-to-day variability. However, more detailed studies with the isotachophoretic technique are required to define the dependence of urinary oxalate on dietary oxalate.

Progress in oxalate research has been hampered to a large extent by the lack of a simple, rapid and accurate method of measuring oxalate in body fluids. Isotachophoretic measurement of oxalate provides a valuable investigational tool for studies of urinary oxalate in metabolic disease, e.g. oxalosis and nephrolithiasis, as demonstrated by the present study. Urinary oxalate was found to be elevated in recurrent renal-stone formers by some [14] but not all [15] investigators and higher urinary oxalate excretion rates were also noted in the present study.

The present study provides clear evidence that isotachophoretic separation of unprocessed urine permits satisfactory measurements of urinary oxalate when the technical details described above are used. It is hoped that isotachophoresis will facilitate further research in this area.

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