Excretion of Urinary Metabolites in Calcium Oxalate Urolithiasis

Effect of Tryptophan and Vitamin B_6 Administration

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APPROXIMATELY two-thirds of human kidney calculi are composed of either pure calcium oxalate or calcium oxalate mixed with apatite.[1] Although considerable clinical observation and research has been devoted to this problem, little is known regarding the cause and prevention of oxalate calculi. Recent studies in this laboratory have associated increased excretion of oxalate in the urine with vitamin B_6 deficiency.[2,3] Renal calculi of calcium oxalate monohydrate have been produced in vitamin B_6 deficient rats[4] and oxalate nephrocalcinosis has been observed in vitamin B_6 deficient cats.[3] In both of these species, the pathologic processes observed resembled their human counterparts. These observations have supplied a new approach to the study of diseases of the kidney, associated with oxalate deposition. The present study was undertaken to compare the urinary excretion of various metabolites, including some associated with vitamin B_6 in normal adults and persons with histories of oxalate renal calculi. The effect of the administration of vitamin B_6 and tryptophan on the excretion of some of these metabolites has also been investigated.

EXPERIMENTAL

The subjects studied were twelve normal adults and eighteen adult patients whose records evidenced recurrent formation of pure calcium oxalate kidney stones. Two twenty-four hour collections of urines or the control samples were obtained from each of these people. The patients were then fed 10 gm. of DL-tryptophan and another twenty-four hour urine specimen was collected. After a lapse of at least two more days, all of the subjects took 2 doses of 20 mg. pyridoxine HCl, orally, twenty-four hours apart and, following the second dose, another twenty-four hour urine collection was made. The samples of urine obtained during each collection period were analyzed for creatinine,[5] xanthurenic acid,[6] oxalic acid,[7] citric acid[8] and 4-pyridoxic acid.[9] The control samples were also analyzed for sodium and potassium (using the method of flame photometry), nitrogen (employing Kjeldahl's method), calcium,[10] magnesium,[11] phosphate phosphorus[12] and chloride.[13] All samples were preserved under toluene and were refrigerated or frozen. During the experimental period, the subjects ate their usual diets. None of the subjects were receiving vitamin therapy before the experimental period began.

RESULTS

The results of the analyses of the control samples of urine are provided in Table 1. Patients with histories of oxalate calculi did not excrete significantly more oxalate than the normal subjects. However, they did excrete significantly more xanthurenic and pyridoxic acids and less citric acid than the normal subjects.

The citric acid excretion values of only

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TABLE I
Twenty-Four Hour Excretion of Metabolites by Normal Adults and Oxalate Calculi Formers (Patients)

Metabolite	Normal Subjects* (12)	Patients* (18)	Statistical Significance†
Creatinine (gm.)	1.53 ± 0.11	1.51 ± 0.10	NS
Xanthurenic Acid (mg.)	6.8 ± 0.6	22.4 ± 5.1	p<0.01
Oxalic Acid (mg.)	37.8 ± 2.4	39.4 ± 2.7	NS
Citric Acid (mg.)	813 ± 60	535 ± 77‡	p<0.01
Pyridoxic Acid (mg.)	3.1 ± 0.3	5.0 ± 0.6	p<0.01
Calcium (mg.)	295 ± 26	377 ± 36	NS
Magnesium (mg.)	79 ± 6	72 ± 5	NS
Nitrogen (gm.)	14.8 ± 2.4	11.3 ± 0.7	NS
Phosphate phosphorus (mg.)	930 ± 49	889 ± 61	NS
Sodium (mEq.)	168 ± 10	191 ± 19	NS
Potassium (mEq.)	65 ± 4	54 ± 5	NS
Chloride (mEq.)	170 ± 15	188 ± 43	NS

* All values include the standard error of the mean.
† NS = not significant.
‡ Includes citric acid values of only twelve patients.

twelve of the patients are included in the table. Six of the patients had had kidney infections and it was felt that the low excretion of citric acid by these persons (mean of 164 mg. per 24 hours) might be, at least in part, the result of bacterial action. Values for the excretion of calcium, magnesium, nitrogen, phosphate phosphorus, sodium, potassium and chloride were not significantly different in the two groups. The nitrogen excretion values for the normal subjects included one high value obtained from a subject who ate a diet with an extremely high protein content. The mean value of the normal group, excluding this person, was 12.4 ± .8 gm. of nitrogen per hour.

In this type of study there are always difficulties in providing adequate control. The diets of the subjects were not the same and the average age of the patients was forty-six years while that of the normal subjects was thirty-one years. However, the almost identical creatinine excretion values obtained are an indication of the similarity in muscle mass of the subjects in both groups. Creatinine values, also were of use as a check on the completeness of individual twenty-four hour urine collections.

Summarized in Table II are the changes from the control values for the urinary excretion of several metabolites after the administration of tryptophan and vitamin B_6. Following the feeding of tryptophan, a significant rise in oxalic acid excretion of the patients occurred. Only three of the normal subjects received tryptophan loads and their oxalic acid excretions increased 12.6, 16.3 and 21.7 mg., respectively. The administration of vitamin B_6 resulted in a decrease in oxalate excretion by all but one of the patients and one of the normal subjects. The latter showed a large increase in oxalic acid excretion. If the value obtained for this person had not been included in the data in Table II, the change in oxalic acid excretion of the normal subjects, following vitamin B_6 administration, would have been −5.4 mg. instead of −3.6 and the difference would have

TABLE II
Changes in Twenty-Four Hour Urinary Excretion of Metabolites Following Tryptophan and Vitamin B_6 Administration

Metabolite	Normal Subjects (10)	Patients (18)	
	Vitamin B_6	Tryptophan	Vitamin B_6
Oxalic acid (mg.)	−3.60 NS*	+9.60 p<0.01	−5.30 p<0.01
Xanthurenic acid (mg.)	−2.00 NS	+24.40 NS*	−6.20 NS
Citric acid (mg.)	−61.00 NS	−28.00 NS	+2.00 NS
Pyridoxic acid (mg.)	+5.20 p<0.001	−0.07 NS	+6.80 p<0.001
Creatinine (gm.)	−0.08 NS	+0.06 NS	+0.13 NS

NOTE: Significance determined by *t* test on the differences between control values and those obtained following the load tests. NS = not significant. * Lack of statistical significance due to one abnormal value in each group (see text).

been significant at less than the 0.01 level.

Changes in xanthurenic acid excretion, following the tryptophan and vitamin B_6 loads, were in the same direction as those obtained for oxalic acid. The mean increase of 24.4. mg. in the xanthurenic acid excretion of the patients was distorted by an increase of 238.8 mg. in the urine of one of the patients. Fifteen of the eighteen patients showed an increased excretion of xanthurenic acid following tryptophan ingestion; however, the extreme value of the one patient, even though it was in the same direction as all but three of the samples, increased the standard error of the mean to a point where the t test did not give a significant difference. If this value had been omitted from the determination of the mean, the change in the xanthurenic acid excretion of the patients would have been +11.8 mg. and the difference would have been significant at less than the 0.01 level. The administration of vitamin B_6 resulted in decreases in the xanthurenic acid excretion of thirteen of the eighteen patients and eight of ten of the normal subjects.

Neither the administration of tryptophan nor of vitamin B_6 had a significant effect on the excretion of citric acid by the subjects; the normal controls continued to excrete considerably more citric acid than the patients during all the collection periods.

Excretion of 4-pyridoxic acid was not affected by the tryptophan feeding and, as expected, increased after the administration of vitamin B_6. Neither of the loads affected the excretion of creatinine significantly.

COMMENTS

Both in this study and a previous study of mentally deficient children,[14] the administration of vitamin B_6 to individuals presumed to be receiving a diet adequate in vitamin B_6 resulted in a significant decrease in oxalate excretion. Although the decrease observed in this work was not large, averaging approximately 15 per cent, it was observed in all but two of the subjects. In areas of the world where people are poorly nourished and among people subjected to nutritional stresses brought about by war, the incidence of kidney disease, associated with oxalate deposition and oxaluria, is increased.[3] The amount of oxalate excreted by these people and the effect of vitamin B_6 on their excretion of oxalate have not yet been studied.

Presumably, the formation of urinary calculi of calcium oxalate must be related to an increased concentration of oxalate or calcium in the urine, to altered solvent characteristics of some urines toward calcium oxalate, or to both. It has not yet been determined whether or not continuous supplementation with vitamin B_6 can bring about a sustained decrease in oxalate excretion. This would be of some importance since it can be argued that the risk of formation of oxalate stones might be lessened if urinary oxalate were decreased.

Until recently, urinary oxalate was generally considered to be almost entirely exogenous in origin. It has now been shown in human beings[15] and animals[2] that much of it may be derived endogenously from glycine. In the study reported herein, the feeding of tryptophan resulted in a rise in oxalate excretion from all but three of the subjects tested. Other studies are needed to determine whether or not the tryptophan acts as an oxalate precursor, or alters the metabolism of the subjects in another way so that more oxalate is produced from other precursors.

Although vitamin B_6 is involved in many fundamental biochemical reactions, methods for the measurement of the adequacy of vitamin B_6 nutriture have not been satisfactory. Intermediates of tryptophan metabolism excreted in the urine, particularly xanthurenic acid which increases in subjects with vitamin B_6 deficiency, are often used as measures. The major metabolite of vitamin B_6 in the urine, 4-pyridoxic acid, has also been used in assessing vitamin B_6 nutriture. Significantly more xanthurenic acid was excreted in control urines from the patients than in those from the normal subjects. The most xanthurenic acid excreted by any of the normal subjects in their control samples was 10.9 mg. per 24 hours. Ten of the eighteen patients showed more than twice the mean for the excretion of xanthurenic acid of the normal subjects, and five of the ten patients excreted more than 30 mg. per 24

hours, an amount which Vilter et al.[16] consider to be abnormal even following a tryptophan load test. In the twenty-four hours after the administration of tryptophan, eleven of the eighteen patients excreted more than 30 mg. of xanthurenic acid, but, following the administration of vitamin B_6, only one person's excretion of xanthurenic acid remained at more than 30 mg. per day. The highest control value for a patient was 71.2 mg. per 24 hours; following the tryptophan load test, she excreted 310 mg. of xanthurenic acid in the twenty-four hour period. This patient was the only one who experienced gastric distress following receipt of the tryptophan load. After administration of vitamin B_6, her excretion of xanthurenic acid in twenty-four hours dropped to 4.6 mg.

Unfortunately, the method commonly used in measuring 4-pyridoxic acid in urine also measures fluorescent substances other than 4-pyridoxic acid. Thus, the values reported for 4-pyridoxic acid are probably somewhat in error. This would be particularly true if the subjects being studied had been consuming diets deficient in vitamin B_6. The people used in this investigation consumed their usual mixed diets which presumably contained 1 to 2 mg. of vitamin B_6 per day (the suggested requirement by the National Research Council).[17] The control samples from the normal subjects contained significantly less 4-pyridoxic acid than did those from the patients. As expected, in both groups there was a marked increase in the excretion of 4-pyridoxic acid following receipt of the vitamin B_6 loads. This increase was slightly greater in the urines of the patients; in the twenty-four hours following the administration of the pyridoxine loads, the normal subjects excreted 8.2 ± 0.8 mg. of 4-pyridoxic acid ($p < 0.01$) and the patients, 11.9 ± 0.9 mg.

It is impossible at this time to properly determine the meaning of the data concerning xanthurenic and 4-pyridoxic acids obtained in this experiment. Certainly, it appears that there are significant differences within the groups in the excretion of these two metabolites associated with vitamin B_6 nutriture. The data concerning xanthurenic acid excretion are particularly interesting because, of the eighteen patients studied, six showed control levels of xanthurenic acid that were less than the mean of the normal subjects, but nine excreted an amount of xanthurenic acid considerably more than twice the highest control value of the normal subjects. If these higher values for excretion of xanthurenic acid can be interpreted as representing a metabolic abnormality, then apparently such an abnormality is not present in all of the patients.

In this and other studies,[18] no significant difference in urinary oxalate excretion has been observed in people, with or without histories of renal oxalate calculi, in well nourished populations. These observations suggest the possibility that the solvent characteristics of urine rather than the quantity of oxalate are primarily responsible for the formation of urinary stones. Miller et al.[19] have shown that calcium oxalate is extremely soluble in urine in comparison to water. They found that citric acid in particular and various electrolytes markedly increased the solubility of oxalate.

In our study, no significant differences were observed in the urinary excretion of electrolytes. However, normal subjects excreted significantly more citric acid than the patients. A number of investigators[20–22] have pointed out that decreased excretion of citrate occurs in subjects with calcium nephrolithiasis. They have suggested that decreased citrate excretion might be of importance in the etiology of calcium calculi. Conway et al.[23] consider any gross diminution of urinary citrate to be due to infection of the urinary tract. We have not included in the tables of this report, the values for the excretion of citric acid of six of the patients with histories of urinary infections. The citric acid excretions of all of these subjects were considerably below the mean of the other patients. Harrison and Harrison[24] have found that calcium phosphate crystals precipitate in the kidneys of rats in which the urinary citrate excretion is inhibited by acetazoleamide. In our laboratory,[4] it has been demonstrated that acidification of the urine (a process which decreases citrate excretion) enhances the development of calcium oxalate concretions in the kidneys of rats with vitamin B_6 deficiency. It seems possible that the formation of oxalate

stones in the people studied could have been caused, in part, by a decreased solubility of oxalate in urine, related to the urinary citrate concentration.

SUMMARY

Studies of the urinary excretion of a number of metabolites by normal adults and by persons suffering from chronic formation of calcium oxalate calculi have been made. The normal subjects excreted significantly less xanthurenic acid and 4-pyridoxic acid and more citric acid than the patients.

Following administration of tryptophan, there was a marked rise in the excretion of oxalate. In all but two of the subjects, ingestion of vitamin B_6 was followed by a decrease in urinary oxalate.

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