The Effects of an Induced Pyridoxine and Pantothenic Acid Deficiency on Excretions of Oxalic and Xanthurenic Acids in the Urine

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UNTIL RECENTLY, urinary oxalic acid was considered to be almost entirely of exogenous origin. It has now been shown in animals and in man that oxalic acid may be derived endogenously from glycine via glyoxylic acid.[1-5] Although the reconversion of glyoxylic acid to glycine, i.e., glyoxylic acid + glutamic acid → glycine + α-ketoglutaric acid, has been shown to be favored over oxalic acid formation,[1] inhibition of this reaction, such as may occur in a lack of pyridoxine, could result in an accumulation of glyoxylic acid with increased oxalic acid production. In support of this postulation are the recent reports that rats and kittens fed pyridoxine-deficient diets had oxaluria and that the addition of glycine and/or desoxypyridoxine to the diets resulted in even greater amounts of urinary oxalic acid.[6-11] In addition, it has been reported that healthy subjects receiving diets thought to be adequate in pyridoxine, excrete decreased amounts of oxalic acid after the ingestion of 10 to 20 mg. of pyridoxine hydrochloride.[12,13]

This experiment was undertaken to study the effects of induced pyridoxine and pantothenic acid deficiency in man with particular reference to the development of immunity.[14] The excretions of oxalic and xanthurenic acids following weekly tryptophan load tests were superimposed on the basic experimental plan.

EXPERIMENTAL

Five apparently healthy men, aged thirty-two to thirty-eight years, participated in a twelve week study. During weeks I and XII, they received weighed, general diets furnishing approximately 3,000 calories and 70 gm. of protein daily. A formula which supplied 70 gm. of protein as vitamin-free casein and contained sucrose, dextrimaltose, cornstarch and corn oil to approximate 3,000 calories was fed via gastric tube during weeks II through XI (weeks 1 through 10 as reported by Hodges et al.[14]). Vitamins and minerals, with the exception of pyridoxine and pantothenic acid were added to the formulas in amounts to meet adult male requirements.[15] During weeks II to VI, inclusive, each man also received 400 mg. of desoxypridoxine and 4 gm. of omega-methyl pantothenic acid daily. The vitamin antagonists were replaced with pyridoxine hydrochloride (600 mg. per day) and calcium pantothenate (2 gm. per day) during weeks VII to XI, inclusive.

Ten grams of D,L-tryptophan suspended in 60 ml. of 2 per cent carboxymethyl cellulose with 0.15 per cent sorbic acid as a preservative, were administered orally or in the formulas on the same day of each week during weeks I through XI. The day tryptophan was given was designated as the "test day"; the previous day and subsequent days were called the "control day" and "following days," respectively. The week of study is designated by Roman numerals. Twenty-four hour urine samples were collected under a preservative made up of 1 part toluene and 5 parts glacial acetic acid. After the sample reached the laboratory, 3 ml. additional acetic acid was added to maintain a suitable hydro-

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gen ion concentration. Aliquots were stored under refrigeration until the time of analysis.

The procedure used for the determination of xanthurenic acid was that published by Glazer et al.[16] with the following adjustments: (1) a 4 per cent sorbitol solution was added as a stabilizing agent because of the tendency for the formation of a precipitate; (2) cloudy urine samples were filtered and all samples adjusted to a hydrogen ion concentration of 7.52 to 7.58 with a Coleman pH meter; and (3) carbon dioxide-free water was used for all dilutions.

The method of Yarbro and Simpson[17] was employed for the determination of urinary oxalic acid. To avoid interference by magnesium, phosphate and sulfate in the determination of urinary oxalate with potassium permanganate, acidified urine should be extracted with ether and the oxalates precipitated from the extract. In this laboratory, the extraction tubes of the Soxhlet apparatus were fitted with overflow tubes and funnels which permitted continuous ether extraction of an acidified urine sample. This apparatus differed from that described in the original method in that it allowed the use of a larger sample of urine. Proportionately larger amounts of reagents were added.

Nitrogen determinations were made on seven day urine aliquots, seven day food aliquots and seven day fecal collections. The boric acid method for Kjeldahl nitrogen was employed.[18] Nitrogen balances were calculated from these determinations.

RESULTS

Physical symptoms related to the deficiencies of pyridoxine and pantothenic acid were noted as early as weeks III and IV.[14] Four subjects followed the experimental plan except that the caloric intakes of Subjects 5A and 4A were increased to 3,200 during weeks III and IV, respectively. Subject 2A complained of epigastric pain and nausea during weeks III and IV and refused or vomited most of his formula. During week V, his caloric intake was decreased to 2,500, but he was unable to retain the entire formula. A weighed, general diet furnishing 2,500 calories was fed during week VI but Subject 2A did not improve clinically until week VII when the vitamin antagonists were replaced with the respective vitamins. During weeks VIII to XII, inclusive, his diet was increased to furnish 3,000 calories and the same amounts of protein, fat and carbohydrate as contained in the basic formula.

According to Hodges et al.,[14] nitrogen losses correlate closely with the occurrence of symptoms of the combined pyridoxine and pantothenic acid deficiencies. Intersubject variation in xanthurenic acid and oxalic acid excretions also may reflect the severity of the combined deficiencies induced in the individual men; hence, the nitrogen retentions and the corresponding excretions of xanthurenic acid and oxalic acid are presented for the successive weeks of the experiment (Fig. 1–4).

During weeks II to VI, inclusive, when the subjects received 400 mg. of desoxypyridoxine and 4 gm. of omega-methyl pantothenic acid daily, xanthurenic acid excretions were increased on the test days. The degree of in-

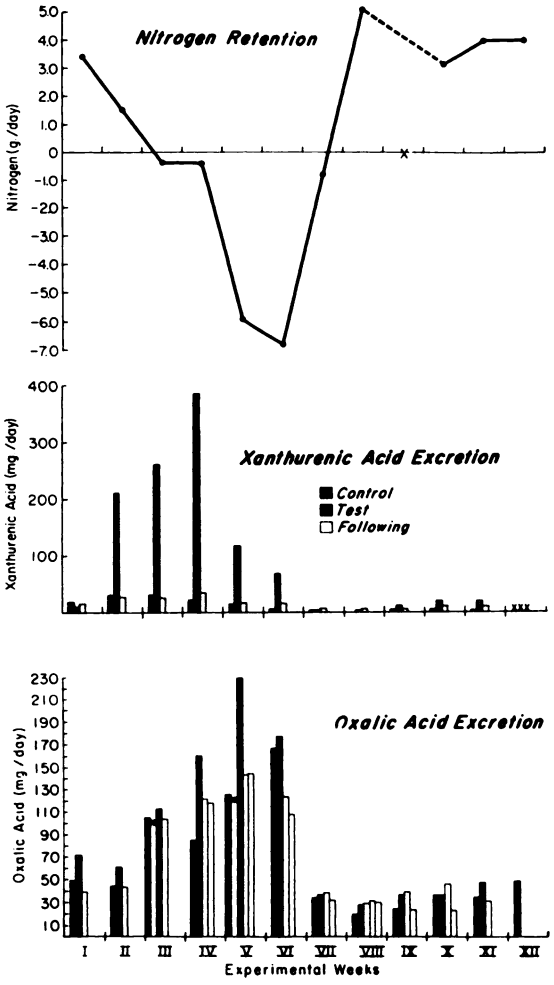

FIG. 1. Excretions of urinary oxalic and xanthurenic acids and nitrogen retentions for Subject 2A.

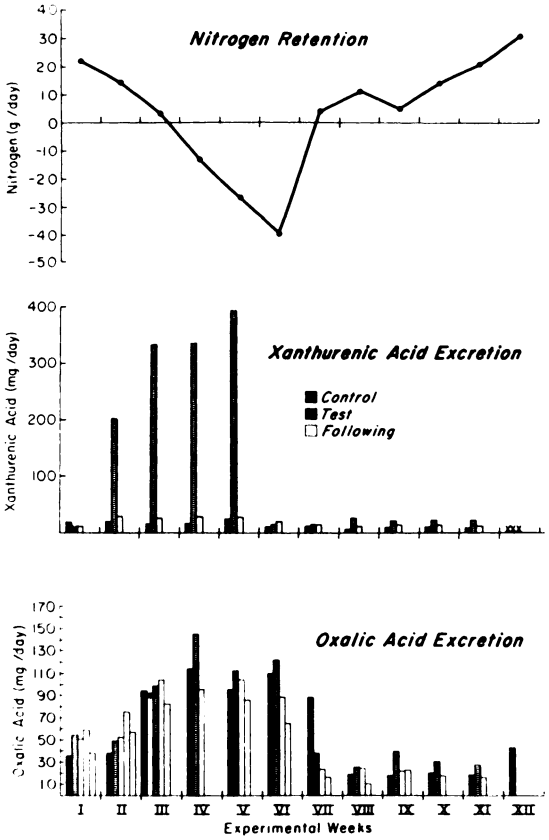

FIG. 2. Excretions of urinary oxalic and xanthurenic acids and nitrogen retentions for Subject 3A.

crease varied with the subject, and in three subjects (2A, 3A and 4A) these elevated excretions were not maintained despite continued daily doses of the antagonists. These subjects were in severe negative nitrogen balance when the decreases in xanthurenic acid excretion occurred. Figures 1 and 2 give examples of the type of response seen. The response of Subject 4A was essentially the same as that for Subject 3A and has not been included. In contrast, Subjects 1A and 5A did not go into severe negative nitrogen balance, and their urinary xanthurenic acid increased progressively with antagonist administration. The data for Subject 1A are given in Figure 3; Subject 5A responded similarly.

Hodges et al.[14] determined xanthurenic acid excretions by the method used by Porter et al.[19] but did not observe a reduction in excretion when the nitrogen balance became negative. It has been recognized that free xanthurenic acid as well as conjugates of this metabolite are excreted in the urine.[20,21] Since the various methods for determining the xanthurenic acid content give similar results with pure xanthurenic acid but not with urine samples, it appears that some methods measure only free xanthurenic acid whereas others measure conjugates of xanthurenic acid also. The method employed herein is thought to measure free xanthurenic acid, an hypothesis presently being tested by chromatographic separation of the compounds excreted in the urine following a tryptophan load test under varying conditions of pyridoxine nutriture.

In man, as in animals, an induced pyridoxine deficiency results in oxaluria. Although the subjects received the formula and vitamin

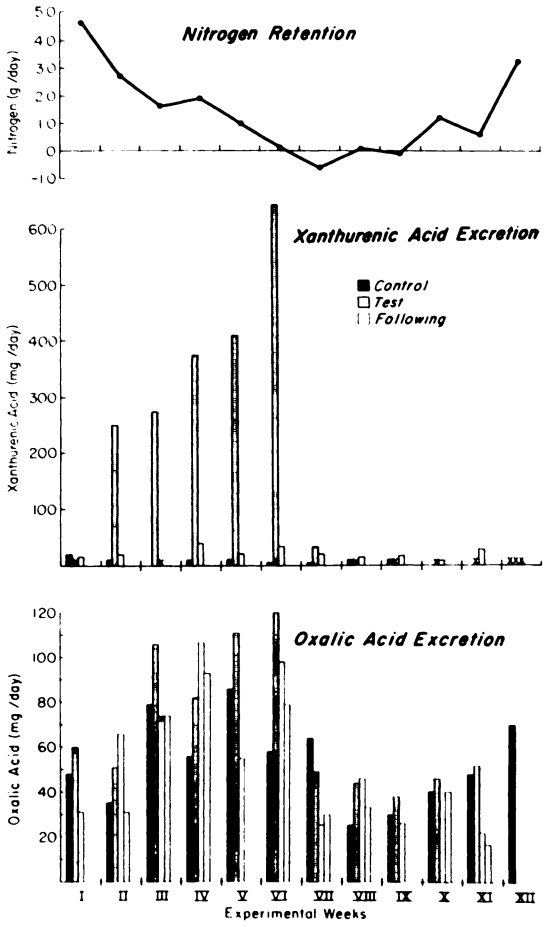

FIG. 3. Excretions of urinary oxalic and xanthurenic acids and nitrogen retentions for Subject 1A.

antagonists one day prior to the control day of week II, urinary oxalic acid excretions did not increase until the control day of week III. During weeks IV to VI, inclusive, urinary oxalic acid increased for all subjects on the control days. The replacement of the vitamin antagonists with the respective vitamins on the control day of week VII did not result in a decrease in oxalic acid excretions on that day; however, on the test day of week VII, a sharp decrease was noted. With continued vitamin supplementation, urinary oxalic acid was excreted in amounts equal to or less than those found in week I.

Subject 2A became the most severely ill during the deficiency period (Fig. 1). His nitrogen retention decreased abruptly during weeks V and VI, and he excreted greater amounts of oxalic acid than did any of the other subjects. During week VI a decrease in oxalate excretion coincided with the replacement of the formula with natural foods, and Subject 2A recovered rapidly from the induced deficiencies with vitamin supplementation in week VII. During weeks VII to XI, inclusive, his oxalate excretions remained below those found during week I.

Nitrogen retentions for Subject 3A also decreased sharply during weeks V and VI (Fig. 2). He excreted greater amounts of oxalic acid than did Subjects 4A, 1A and 5A. With vitamin supplementation, recovery from the combined deficiency was rapid, and his oxalate excretions were below those measured during week I.

Subject 4A responded similarly to Subject 3A except that decreases in nitrogen retention were not as abrupt as those for Subjects 2A and 3A, although he lost nitrogen throughout the deficiency period. Oxalic acid excretions also decreased more gradually following vitamin supplementation in Subject 4A.

Although Subject 1A lost nitrogen during the period of deficiency, he did not go into severe negative nitrogen balance. His oxalic acid excretions increased during weeks II to VI, inclusive, and then decreased to equal those found during week I (Fig. 3). Subject 5A excreted the least amounts of oxalic acid of any of the subjects. He did not lose nitrogen and exhibited only mild symptoms of the combined vitamin deficiency.

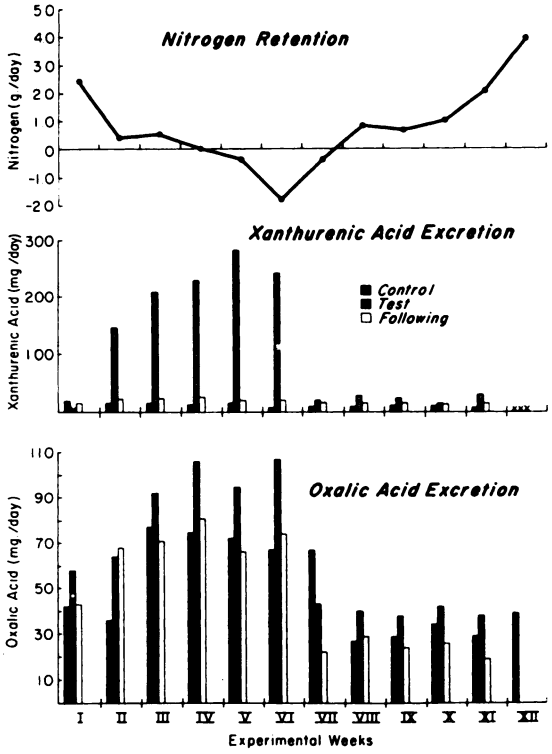

FIG. 4. Mean excretions of urinary oxalic and xanthurenic acids and mean nitrogen retentions for Subjects 3A, 1A, 4A and 5A.

Although intrasubject and intersubject variation[22–25] in urinary oxalic and xanthurenic acids was noted, group trends in excretions did parallel changes in dietary and treatment regimens. Figure 4 presents the mean xanthurenic acid and oxalic acid excretions and nitrogen retentions for Subjects 1A, 4A, 3A and 5A. Subject 2A was omitted from the averages as he received the formula feeding only for weeks II through V. His condition improved somewhat on a general diet although neither the symptoms of deficiency nor the biochemical responses returned to pretreatment levels until the antagonists were replaced by the respective vitamins.

COMMENT

Numerous investigators have related urinary xanthurenic acid excretions to pyridoxine

nutriture,[14,16,26-29] and in animals, an induced pyridoxine deficiency is associated with increased oxalic acid synthesis and oxaluria. There is no known reason to assume that the concurrent deficiency of pantothenic acid would influence the formation of oxalic or xanthurenic acids although it is recognized that the effect of the combined deficiency is unknown.

The discussion of xanthurenic acid excretion will be related to those studies in the literature which employed the same method of analysis. Vilter and associates[29] studied fifty patients who received desoxypyridoxine. Of these, thirty-four had clinical symptoms of pyridoxine deficiency, and thirty excreted 56 to 500 mg. of xanthurenic acid following a tryptophan load test. Vilter considered an excretion of over 30 mg. of xanthurenic acid after administration of a tryptophan load to be indicative of pyridoxine deficiency. Xanthurenic acid usually increased in the urine before symptoms were manifested.

Similar data were found with our subjects except for the one instance when only 16 mg. of xanthurenic acid was excreted on a test day (Subject 3A) despite prolonged desoxypyridoxine administration. This marked decrease in urinary xanthurenic acid and the less pronounced decreases noted for Subjects 4A, 3A and 2A, when they were in severe negative nitrogen balance, are of interest. Biehl and Vilter[28] reported xanthurenic acid values after tryptophan load tests for subjects with an isonicontinic acid hydrazid-induced pyridoxine deficiency. The test day excretion of half their subjects increased at the beginning of the period of pyridoxine deficiency and then decreased unexpectedly despite continued administration of vitamin antagonist. It is not known if these subjects were in negative nitrogen balance; however, decreased nitrogen retention has been reported as a characteristic of pyridoxine deficiency.[30-32]

Loss of nitrogen during the period of pyridoxine deficiency appears to be primarily due to tissue catabolism. It is possible that tissue catabolism releases pyridoxine. Since xanthurenic acid values measured with the method of Porter et al. did not decrease,[19] it might be concluded that tryptophan metabolism adapted, or shifted, to pathways which more easily form xanthurenic acid conjugates.

Slight decreases in oxalic acid excretions were found for Subjects 3A and 2A on the test day of week VI; however, these decreases were of a degree which could reflect intrasubject variation. Those subjects with decreased nitrogen retentions throughout the period of deficiency had increased oxalic acid excretions and manifested more severe deficiency symptoms. This relationship probably only emphasizes the fact that deranged protein metabolism and oxalic acid production simultaneously reflect the severity of the induced pyridoxine deficiency. If there is pyridoxine released from tissue catabolism, it appears to be preferentially utilized for tryptophan metabolism. When the vitamins were restored, there was a delay of approximately a day in the return of the oxalic acid excretions to that found in week I as compared with the correction of the xanthurenic acid excretion, suggesting again that the vitamin was used preferentially to metabolize the tryptophan load.

SUMMARY

Five healthy, adult men were made pyridoxine and pantothenic acid-deficient by use of a semisynthetic formula and desoxypyridoxine and omega-methyl pantothenic acid supplements. In addition to the weekly determination of nitrogen retention, the urinary excretions of xanthurenic and oxalic acids were studied before and after a test dose of D,L-tryptophan given weekly during the twelve week study. Pyridoxine and pantothenic acid were restored to the diet after the seventh week.

In man, as in animals, an induced pyridoxine deficiency results in oxaluria. Following the administration of test doses of tryptophan to pyridoxine-deficient subjects, the amounts of urinary oxalic acid were sharply increased for one to two days.

Excretion of xanthurenic acid increased after each test dose of tryptophan until the fifth week of study. At that time, three men became negative in nitrogen balance abruptly, and excretion of xanthurenic acid decreased to about half that observed the previous week. Two men did not lose nitrogen, and the trend of

excretion of the metabolite did not change until pyridoxine was restored to the diet. In contrast, those subjects with decreased nitrogen retention excreted the greatest amounts of oxalic acid. It is postulated that catabolism of tissue may release sufficient pyridoxine to metabolize the tryptophan load, in part, but that preferential use of the vitamin must occur.

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