Is the Tryptophan Load Test a Valid Index for a Chemically-Induced Vitamin B_6 Deficiency? (31482)

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In an earlier communication it was reported that aminooxyacetic acid (AOAA) was a convulsant when employed in high doses(1). It was postulated that the toxicity associated with high doses of AOAA was due to oxime formation between AOAA and the aldehydic form of vitamin B_6 which rendered the vitamin inactive. Subsequent studies showed that chronic administration of AOAA to rats maintained on a diet containing vit. B_6 resulted in a deficiency of the vitamin as indicated by the elevation of urinary xanthurenic acid excretion after tryptophan loading(2). However, the validity of increased xanthurenic acid excretion after tryptophan as a test for vit. B_6 deficiency has been questioned since it is an indirect test, and it was suggested that large quantities of tryptophan could result in an upset of intermediary amino acid balance (3). It was advocated that direct metabolites of vit. B_6 such as 4-pyridoxic acid be measured when studying conditions implicating a vit. B_6 deficiency(4). The purpose of this study was to establish whether or not the tryptophan load test did indeed reflect a vit. B_6 deficiency induced by AOAA. Direct tissue measurements of pyridoxal phosphate and pyridoxamine phosphate and two indirect indices, namely oxalic acid excretion and pyridoxic acid excretion, were measured concomitantly with xanthurenic acid excretion in order to accomplish this end.

Methods. Female Sprague-Dawley rats weighing approximately 200 g were placed singly in metabolism cages and allowed to adjust to their new environment and to become accustomed to tube feeding. After 3-4 days, all animals were fed by stomach tube 10 ml, twice daily, of semisolid diet prepared by homogenizing in a Waring blender 300 g of test diet L-462 (General Biochemicals) in 450 ml water. The composition of the diet is shown in Table I. In addition, 10 mg/kg of L-tryptophan were given i.p. except in one experiment in which tryptophan was omitted.

TABLE I. Dietary Ingredients.

Ingredients	g/kg
Whole wheat flour	318.0
Yellow corn meal	98.1
Quaker rolled oats	212.0
Lactalbumin	106.0
Casein, plain	53.0
Whole milk powder	106.0
Alfalfa leaf meal	21.2
Desiccated liver	21.2
Calcium carbonate	11.66
Ladek	
Vitamin A concentrate, 200,000 U/g	.04244
" D_3 Dawe's sterol, 1500 U/g	.70734
" E, mixed tocopherols, U/g	.39788
Menadione	.10610
Corn oil	20.77394
Salts (modified)	
Sodium chloride	5.4409
Potassium iodide	.0477
Magnesium sulfate	4.7639
Manganese sulfate mono.	.6122
Ferric citrate	4.7639
Cupric sulfate	.2377
Cobalt chloride	.0318
Zinc sulfate	.0403
Sodium fluoride	.0159
Sodium borate	.0318
Aluminum potassium sulfate	.0477
Molybdenum trioxide	.0318
Magnesium acetate	10.0796
Thiamine HCl	.0530
Ascorbic acid	1.0610
Vitamin B mix	
Thiamine HCl	.0318
Riboflavin	.0159
Nicotinamide	.0275
Nicotinic acid	.0275
Calcium pantothenate	.1591
Choline chloride	1.0610
Pyridoxine HCl	.0106
Pyridoxamine DiHCl	.0021
Biotin	.0005
Folic acid	.0053
Para-aminobenzoic acid	.0265
Vitamin B_{12} with mannitol, 0.1% trituration	.1326
i-Inositol	.5305
Corn starch	1.1539

This treatment was continued for a period of 4-7 days before commencing drug treatment of 10 mg/kg of AOAA, i.p., twice daily. Twenty-four hour urine samples were collected under toluene and their pyridoxic,

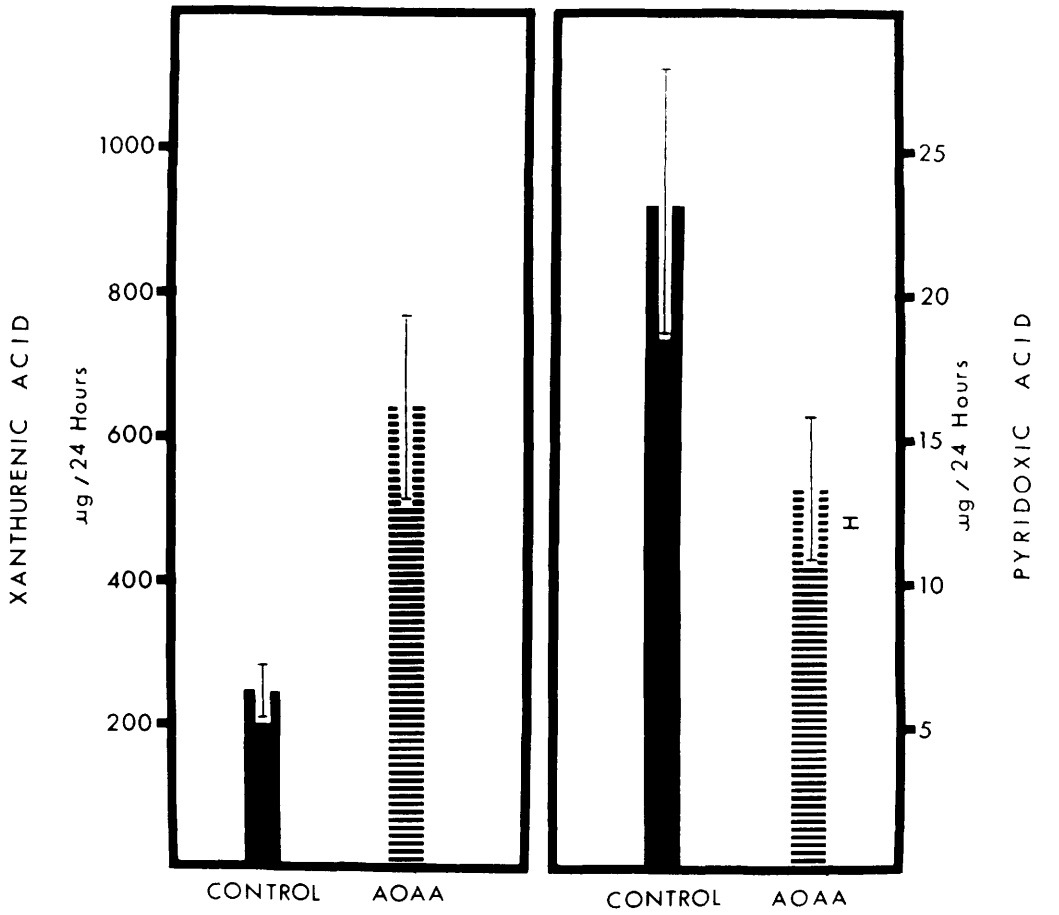

FIG. 1. 4-day averages (mean ± s.d. of daily excretion of xanthurenic and pyridoxic acids before and after aminooxyacetic acid (10 mg/kg i.p., twice daily).

xanthurenic and oxalic acid contents were determined.

Pyridoxic acid was assayed by the spectrophotofluorometric method of Reddy *et al*(5), the method of Glazer *et al*(6) was used for the xanthurenic acid and that of Powers and Levatin(7) for oxalic acid.

In the studies dealing with the tissue pyridoxal phosphate and pyridoxamine phosphate, rats were given 10 mg/kg of AOAA i.p. twice daily for 2 days. They were sacrificed on the third day one-half hour after receiving a single AOAA dose of 20 mg/kg i.p. The brain and liver were rapidly removed and homogenized in 5 volumes of distilled water. Homogenates were then boiled for 5 minutes, centrifuged and suitable aliquots were taken for the determinations, carried out according to the method of Holzer and Gerlach(8).

Results. Following AOAA administration, an immediate reduction (52%) in pyridoxic acid was observed and the daily excretion remained low while the animals were on drug. On the other hand, only a mild xanthurenuria was observed the first day after AOAA treatment, whereas an approximate 3-fold increase in xanthurenic acid excretion over control levels was observed on the following 3 days. Six rats were used in this study. Their average daily urinary output of xanthurenic and pyridoxic acid during the 4-day control period was compared with that of the 4 days while on drug. These results are shown in Fig. 1.

The results of experiments dealing with oxalic acid excretion before and after AOAA treatment are tabulated in Table II. In the

TABLE II. Oxalic Acid Excretion in Rats Before and After Aminooxyacetic Acid (AOAA).

Treatment	Dose (mg/kg, i.p.)	No. rats	Oxalic acid (mg/24 hr)	P
Control period (tryptophan alone)	10	4	.71 ± .11	
AOAA plus tryptophan	10 twice daily 10		1.20 ± .07	<.02
Control period (no treatment)	—	4	.70 ± .05	
AOAA	10 twice daily		1.99 ± .29	<.01

first experiment in which injections of L-tryptophan were given, AOAA administration resulted in a 69% increase in oxalic acid excretion. In a separate study in which conditions were kept identical with all other experiments except that tryptophan loading was omitted, a more marked rise (184%) was observed. The effect of chronic administration of AOAA on the pyridoxal phosphate and pyridoxamine phosphate levels in rat tissues is shown in Table III. A significant reduction in the combined content of both forms of the vitamin after AOAA was observed in the liver but not the brain.

Discussion. Urinary excretion of pyridoxic acid has been shown to reflect the amount of vit. B_6 intake(9). It is formed from pyridoxal and is the metabolic end product of all 3 forms of vit. B_6. It was not unexpected, therefore, that the administration of AOAA resulted in reduced pyridoxic acid excretion, especially since it has already been demonstrated that oxime formation between AOAA and pyridoxal occurs very readily(2). In light of the findings that pyridoxic acid excretion was indeed reduced under the influence of AOAA, it would appear that the oxime produced by the interaction of AOAA and pyridoxal is metabolized at a slow rate.

TABLE III. Tissue Levels of Pyridoxal Phosphate and Pyridoxamine Phosphate After Aminooxyacetic Acid.*

	No. rats	Liver	Brain
		μg pyridoxal-P + pyridoxamine-P/g	
Controls	10	6.45 ± .79	2.71 ± .17
Treated	10	4.60 ± .51	2.42 ± .07
P		<.001	<.2

* The treated rats received 10 mg AOAA/kg, i.p., twice daily, for 2 days and were given 20 mg/kg, i.p., one-half hour before they were sacrificed on the third day.

This is unlike what is apparently the case with the oxime produced by the interaction of hydroxylamine and pyridoxal(10). The decrease in pyridoxic acid excretion observed in these studies was accompanied by an increase in urinary xanthurenic acid, which is in good agreement with data previously reported, using a diet with a different composition.

A rise in urinary oxalic acid has been employed by Gershoff and Faragella(11), as an index for vit. B_6 deficiency. In our studies we observed an approximate 50% increase in this urinary metabolite in the AOAA-treated animals. The possibility exists that AOAA itself was oxidized to oxalic acid and was responsible for at least a part of the observed increase. Since Gershoff and Prien (12) reported that tryptophan loading in itself resulted in a rise in oxalic acid excretion in certain human subjects, we omitted the tryptophan load in one group of rats. Surprisingly, there was a more marked increase in oxalic acid excretion by AOAA when the tryptophan was omitted from the regimen. Tryptophan or one of its metabolites under these conditions thus appears to be inhibiting the formation or stimulating the metabolism of oxalate.

In the subconvulsive doses employed in these studies, administration of AOAA resulted in a partial depletion of liver pyridoxal phosphate and pyridoxamine phosphate. Interestingly, a significant reduction of these constituents was not observed in the brain.

Our results suggested that repeated subconvulsive doses of AOAA were capable of causing a vit. B_6 deficiency and depleting the liver store of its phosphate ester, which is the active cofactor in a number of metabolic reactions. AOAA administered *in vivo* may be related to the inhibition of these pathways. A number of these pathways have been shown

to be inhibited by AOAA *in vitro*(13-16) and in certain cases it appeared that the inhibition was mediated through the aldehydic form of vit. B_6(15,17) findings which are in good agreement with our original postulation that AOAA toxicity was related to its chemical interaction with pyridoxal. Our present studies indicate also that in the case of AOAA-induced vit. B_6 deficiency, all 3 urinary metabolites studied may serve as valid indices for a vit. B_6 deficiency.

Summary. Chronic administration of aminooxyacetic acid (AOAA) to rats after a tryptophan load resulted in an increase in xanthurenic acid and oxalic acid excretion and a decrease in pyridoxic acid excretion, thus suggesting a vitamin B_6 deficiency. Subconvulsive doses of the drug resulted in a partial depletion of liver, but not brain, pyridoxal phosphate and pyridoxamine phosphate. Thus, it would appear that increased xanthurenic acid excretion ofter a tryptophan load is a valid index for measuring a vitamin B_6 deficiency induced by chronic administration of aminooxyacetic acid.

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Progesterone Biosynthesis in Perfused Corpora Lutea.* (31483)

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Studies on metabolism of the ovary indicated that it would be useful to have an *in vitro* system which resembled the *in vivo* corpus luteum with regard to retained anatomical structure, blood supply and compartmentalization. The *in vitro* system was accomplished by isolating the luteal phase ovary and perfusing it through the ovarian artery with a blood substitute(1). The blood substitute was used to reduce or remove the organ from luteotropins and other substances present in the blood which regulate ovarian metabolism. Organs perfused in this manner were found to utilize oxygen for several hours and incorporate acetate-1-C^{14} into ether soluble material(1). This report offers evidence that the labeled acetate in the perfusate was also incorporated into progesterone.

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