PRODUCTION OF THIAMINE DEFICIENCY DISEASE BY THE FEEDING OF A PYRIDINE ANALOGUE OF THIAMINE

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(Received for publication, May 6, 1943)

The production of bacterial growth inhibitors derived from acidic vitamins by the substitution of a sulfonic acid group for the carboxyl group of the vitamin is now well known. Thus sulfanilamide, the sulfur analogue of p-aminobenzoic acid (1), pyridine-3-sulfonic acid, the analogue of nicotinic acid (2), and thiopanic acid, the analogue of pantothenic acid (3-5), have been shown to prevent growth of certain microorganisms. Since addition of the analogous vitamin to cultures inhibited by one of these compounds restored growth, it seemed that the sulfonic acids caused failure of growth by producing deficiencies of the growth factors structurally related to them. However, these three sulfur analogues did not cause deficiency diseases when fed to animals (6, 7). The only deficiency diseases thus far produced in animals by the feeding of structurally similar compounds have been the hypoprothrombinemia caused by administration of 3,3'-methylenebis[4-hydroxycoumarin] (related to vitamin K) (8), and the scurvy-like disease caused by glucoascorbic acid. Since a study of the action of these inhibitors related to the vitamins may provide a key to the study of the mode of action of the vitamins, an attempt has been made to discover such inhibitors derived from some of the other vitamins.

The work of Erlenmeyer (9) suggested that physiological activity was partially retained in compounds in which sulfur in a ring system was interchanged with —CH:CH—. Others (10, 11) have endeavored to show that such an alteration in nicotinic acid (to yield thiazole-5-carboxylic acid) and in thiamine (to yield 2-methyl-4-amino-5-pyrimidylmethyl-(2-methyl-3-hydroxyethyl)pyridinium bromide) would result in compounds with vitamin action. However, Robbins (12) has shown recently that the latter substance, the pyridine analogue of thiamine, had no growth factor action, and actually inhibited growth of certain fungi.

It has now been observed that the feeding of 2-methyl-4-amino-5-pyrimidylmethyl-(2-methyl-3-hydroxyethyl)pyridinium bromide to mice maintained on an adequate diet caused a fatal disease, with many of the characteristic symptoms of thiamine deficiency as seen in other species. For the sake of brevity, the pyridine analogue of thiamine has been named

DISEASE FROM PYRITHIAMINE

pyrithiamine. The disease produced by pyrithiamine administration was prevented or cured by the giving of sufficient amounts of thiamine. When mice were fed a ration free of thiamine, no characteristic symptoms, such as are seen in other species, were observed (13). The animals merely ate less and less, lost weight, and died. However, with administration of pyrithiamine, many characteristic symptoms of thiamine deficiency were seen in all animals. By variation of either the amount of thiamine or of pyrithiamine fed, it was found that about 40 molecules of pyrithiamine would nullify the effect of 1 molecule of thiamine. Hence, pyrithiamine was one of the most active vitamin antagonists thus far studied.

EXPERIMENTAL

Materials and Methods—Pyrithiamine was synthesized according to the directions of Tracy and Elderfield (14). It was administered by giving 0.02 cc. of a solution of desired concentration orally three times daily. Each dose during the day was 4 hours removed from the previous one.

Weanling mice (approximately 12 gm.) were kept in individual cages equipped with screen bottoms and fed the following ration: sucrose 76 gm., vitamin-free casein 18 gm., salts (15) 5 gm., fortified corn oil (16) 1 gm., thiamine 80 γ, riboflavin 500 γ, nicotinic acid 10 mg., pyridoxine 200 γ, calcium pantothenate 2 mg., choline 10 mg., and inositol 100 mg. Such a ration was previously found adequate for mice (17). Each mouse ate about 2 gm. of this ration per day, and hence received 1.6 γ of thiamine. In the later experiments of this series, thiamine was omitted from the ration and administered orally once daily. The mice were weighed twice weekly.

Production of Disease—When sufficient pyrithiamine was given, the following sequence of events was observed in all animals with great regularity. Similar symptoms were seen to appear in every member of a group within 24 hours from the time the first mouse was affected. The animals failed to grow or lost weight, but there was no severe anorexia such as was seen in mice fed a thiamine-free ration. On the 4th or 5th day, they became very inactive and assumed a hunched posture. Then tremors and occasionally convulsions appeared, which were particularly marked when the animals were picked up by the tail. Somewhat later, spasticity, especially of the legs, appeared. Soon it was noted that when the mice attempted to stand erect on their hind legs, they would fall over backwards.

2 A part of the 2-methyl-3-hydroxyethylpyridine used in the synthesis was supplied by Dr. M. Rubin of Wallace and Tiernan Products, Inc. The 2-methyl-4-amino-5-bromomethylpyrimidine hydrobromide used in the synthesis was donated by Dr. T. Jukes of Lederle Laboratories, Inc., and Dr. J. C. Keresztesy of Merck and Company, Inc. We wish to thank these men for their cooperation.
Violent jerking of the head backwards was also seen. Then the legs became weak and unable to support the body. This weakness always appeared on one side first and caused the animals to walk in circles. Following this, both sides became affected. Finally, all legs were affected to such an extent that the animals assumed a characteristic pose, lying on their bellies with the legs spread out on each side at right angles to the body. Shortly thereafter (within 24 hours) they died. No mouse survived longer than 3 days from the onset of symptoms. Data regarding responses to various dose levels of pyrithiamine are summarized in Table I. Except where otherwise stated, the experimental period was 2 weeks.

_Cure and Prevention of Disease with Thiamine_—A group of five mice was given 20 mg. of pyrithiamine daily until they had reached the stage at which they were unable to stand erect on their hind legs. The pyrithiamine treatment was continued and in addition one was given a single dose of 1 mg. of thiamine orally, two were given 500 γ each, and two were kept for controls. Each had lost 2.5 gm. in the 2 days prior to treatment. In all treated animals, improvement was noted within 1 hour, and within 20 hours they were without discernible symptoms. In the 24 hours following treatment, they had gained 1.5, 1.5, and 1.6 gm. The two which were not treated died within 2 days. No more thiamine was given to the treated animals, and within 5 days from the time of treatment they again developed symptoms and died.

Four mice were given 2 mg. daily of pyrithiamine until they were unable to stand. Two were then given 20 γ of thiamine each. The treated mice improved noticeably, but on the following day became worse again and soon died.
Prevention of the disease with thiamine was demonstrated in two experiments. In one, a group of eight mice received 2 mg. of pyrithiamine and 60 γ of thiamine per mouse per day. Appropriate control groups receiving no treatment and pyrithiamine alone were also included. These controls behaved similarly to comparable groups in the previous experiments; that is, mice in the group without pyrithiamine gained an average of 3.0 gm. per week and remained free of symptoms, while those in the group given pyrithiamine developed symptoms, lost weight, and all died within 9 days from the start of the experiment. In the second experiment, the mice received 600 γ of pyrithiamine and 61.6 γ of thiamine per day. Both experiments were continued for 17 days. The pertinent data are summarized in Table I. It can be seen that thiamine protected against the disease.

The effect of pyrithiamine was in some respects delayed and cumulative. Thus when three mice were given 1.2 mg. of pyrithiamine on each of the first 3 days of the experiment, and were then continued on 2 γ of thiamine per day, but without any more pyrithiamine, they grew well and appeared normal for 6 days. On the 7th day, they began to lose weight precipitously and to show the characteristic symptoms evoked by pyrithiamine. On the 9th day, one was treated with a single dose of 500 γ of thiamine. This produced remission of the symptoms within 24 hours. It was of interest that the symptoms appeared in this group almost as rapidly as in the group which had received 1.2 mg. of pyrithiamine every day (see Table I). Very small doses of pyrithiamine given daily for extended periods produced characteristic symptoms eventually. Thus in two experiments when eight mice were given 20 γ of pyrithiamine and 2 γ of thiamine per day, they grew as rapidly as controls which received no pyrithiamine, and remained without symptoms for 2½ weeks. Following this, they ceased to grow, lost weight, and developed characteristic symptoms. These symptoms were promptly cured by a single dose of 1 mg. of thiamine. In a group of three mice which received 20 γ of pyrithiamine and 2 γ of thiamine per day for 14 days, and then 2 γ of thiamine a day but no pyrithiamine, symptoms developed at the 19th day and two of the mice died. The third mouse showed occasional violent fits of jumping, but slowly improved and survived for 4 weeks, at which time the experiment was discontinued.

The apparent delayed and cumulative effect of pyrithiamine made it difficult to establish an exact ratio of thiamine to pyrithiamine. From the curative tests it was seen that thiamine at one-fortieth the level of pyrithiamine would cure the disease. However, as little as 20 γ of pyrithiamine per day in the presence of 2 γ of thiamine per day eventually produced symptoms and death, even though growth was not affected in the early part of the period.
SUMMARY

The feeding of pyrithiamine, the pyridine analogue of thiamine (2-methyl-4-amino-5-pyrimidylmethyl-(2-methyl-3-hydroxyethyl)pyridinium bromide), to mice caused the appearance of characteristic symptoms of thiamine deficiency. The disease was prevented or cured by sufficient amounts of thiamine.

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