# Original Communications

## Factors Affecting Human Antibody Response

### IV. Pyridoxine Deficiency

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A DEFECT in antibody synthesis has been demonstrated repeatedly in animals as a result of pyridoxine deficiency.[1,2] Experimentally induced human pyridoxine deficiency, however, seemed not to interfere with antibody production.[3]

In our search for factors which might interfere with antibody production we decided to repeat Vilter's studies in man. This was particularly desirable because the next step which we planned was induction of a simultaneous deficiency of pyridoxine and pantothenic acid in human volunteer subjects.

#### METHODS AND MATERIALS

Six healthy men from the Iowa State Prison volunteered for the study. They ranged in age from twenty-two to twenty-six years. They were admitted to the Metabolic Ward of the University Hospitals where they spent the following three months. After a complete medical history and physical examination, the men were divided into three groups designated as "control," "deficient" and "antagonist." During the first five weeks the studies were conducted in order to measure gastrointestinal responses to secretin and other substances. At the beginning of the sixth week of hospitalization the present study was begun. The men were given a basic formula similar to that used in previous vitamin studies (Table I). This formula was administered by nasogastric tube twice daily. It supplied adequate calories, protein and all essential nutrients except the one under study. The first pair of men received a complete formula including pyridoxine. The other four men received the same diet without pyridoxine. Two of these men were given, in addition, the anti-vitamin desoxypyridoxine, 100 mg. parenterally and 200 mg. orally each day. In order to determine what effects pyridoxine deficiency might have upon cholesterol metabolism a portion of the fats and proteins was provided by egg yolk in the diet of three men, one from each pair.

Blood was drawn weekly for measurement of serum cholesterol, serum proteins, hematocrit and erythrocyte sedimentation rate. Routine examinations of the urine and routine blood counts were performed each week. On one day of each week the men were given 10 gm. of D,L-tryptophan orally in order to measure the amount of xanthurenic acid excreted in their urine.

All the men had been immunized previously with tetanus and typhoid antigens at the time of admission to the prison, and three of the men had been immunized while in military service. Re-immunization was given with 0.5 ml. of commercial tetanus and 0.5 ml. of commercial typhoid antigens at the beginning of the ninth, tenth and eleventh weeks. Antibody titers were measured every two weeks by methods described previously.[4]

#### RESULTS

The men tolerated the formula well. The

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TABLE I

*A. Ingredients of Formulas*

| Ingredient* | Diet 1 | Diet 2 |
|---|---|---|
| Egg yolk (gm.) | 49 | 0 |
| Casein (gm.) | 66 | 76 |
| Peanut oil (gm.) | 116 | 133 |
| Cornstarch (gm.) | 75 | 75 |
| Dextri Maltose (gm.)† | 100 | 100 |
| Sucrose (gm.) | 207 | 207 |
| Vitamin mix (units) | 1 | 1 |
| Mineral mix (units) | 1 | 1 |

*B. Nutrients Provided by Diet*

| Nutrient | Diet 1 | | Diet 2 | |
|---|---|---|---|---|
| | From Food | From Supplements | From Food | From Supplements |
| Protein (gm.) | 70 | ... | 70 | ... |
| Fat (gm.) | 133 | ... | 133 | ... |
| Carbohydrate (gm.) | 381 | ... | 380 | ... |
| Calcium (mg.) | 170 | 230 | 101 | 299 |
| Phosphorus (mg.) | 352 | 248 | 30 | 570 |
| Iron (mg.) | 4.1 | 4.9 | 1 | 8 |
| Sodium (mg.) | 43 | 2,447 | 10 | 2,490 |
| Potassium (mg.) | 211 | 1,789 | 150 | 1,850 |
| Iodine (μg.) | 9 | 91 | ... | 100 |
| Magnesium (mg.) | 8 | 242 | 1 | 249 |
| Vitamin A (I.U.) | 1,766 | 1,734 | Trace | 3,500 |
| Vitamin D (I.U.) | 83 | 267 | Trace | 350 |
| Vitamin E (mg.) | 3 | 2 | ... | 5 |
| Vitamin K (mg.) | Trace | 1 | ... | 1 |
| Ascorbic acid (mg.) | ... | 30 | ... | 30 |
| Thiamine (μg.) | 160 | 740 | 10 | 890 |
| Riboflavin (μg.) | 220 | 980 | 40 | 1,160 |
| Niacin (mg.) | Trace | 10 | ... | 10 |
| Pantothenic acid (μg.) | 3,310 | 1,690 | 10 | 4,990 |
| Folic acid (μg.) | 15 | 185 | 2 | 198 |
| Biotin (μg.) | 29 | 121 | ... | 150 |
| Pyridoxine (μg.)‡ | 212 | ... | 49 | ... |
| Vitamin $B_{12}$, (μg.) | 0.65 | 0.35 | 0.227 | 0.773 |
| Cystine | 0.39 | 0.40 | 0.28 | 0.30 |
| Methionine | 2.19 | 0.74 | 2.26 | ... |
| Tryptophane | ... | ... | 0.67 | ... |

*C. Mineral Mix (gm.)*

| Mineral | Diet 1 | Diet 2 |
|---|---|---|
| Anhydrous $Mg(OH)_2$ | 127.60 | 131.400 |
| K I | 0.026 | 0.029 |
| $FeC_6H_8O_7 \cdot 3H_2O$ | 5.762 | 9.428 |
| $CaH_4(PO_4)_2 \cdot H_2O$ | 222.022 | 413.636 |
| Ca Lactate $\cdot 5H_2O$ | 117.700 | ... |
| $NaH_2PO_4 \cdot H_2O$ | ... | 105.705 |
| Na Cl | 1,368.673 | 1,347.898 |
| K Cl | 750.734 | 776.108 |

* Cystine was added to provide a tryptophane to sulfur-containing amino acid ratio approximately 1:4.
† Mead Johnson, No. 1.
‡ One man of three (controls) received 2 mg. pyridoxine per day.

TABLE II
Symptoms and Signs

| Week | Control Subjects | | Deficient Subjects | | Antagonist Subjects | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | Initial examination | Initial examination | Initial examination | Initial examination | Initial examination | Initial examination |
| 2 | ... | ... | ... | ... | ... | ... |
| 3 | ... | ... | ... | ... | ... | ... |
| 4 | ... | ... | ... | ... | ... | ... |
| 5 | ... | ... | ... | ... | ... | ... |
| 6 | Formula | Formula | Formula | Formula | Formula | Formula |
| 7 | Mild fatigue | ... | Abdominal uneasiness, headache, skin slightly dry | Mild headache | 4 loose stools per day | Dry mouth |
| 8 | Sleepy, slight headache | ... | Nausea and epigastric burning, abdominal distress | "Butterflies in stomach," unable to concentrate, dizzy, irritable, nausea | Itching and redness of scrotum, shower of dandruff, mild cheilosis, irritable | Sore lips, tires easily, irascible, marked cheilosis, nausea and vomiting |
| 9 | ... | ... | Aching joints, abdominal distress | Flatus and belching, abdominal distress | Mild nausea and vomiting, epistaxis, more nausea, headache, severe diarrhea, foul breath, scrotum scaling | Tongue and lips sore, more nausea and vomiting |
| 10 | ... | ... | Mild cheilosis | Abdominal cramps and flatus, mild | Mild nausea and vomiting | Tongue and lips worse, face dry and scaling, tongue and lips, sore, purple, inflammed, more nausea and vomiting |
| 11 | Vitamins restored, vomited once | Vitamins restored | Vitamins restored, mild hemorrhoids | Vitamins restored | Vitamins restored | Vitamins restored, headache, nausea and vomiting, dizzy, abdominal distress, rectal burning, skin dry and scaling, breath foul, tongue and lips sore, gums bleeding, perineum inflamed |
| 12 | ... | ... | ... | Mild gastroenteritis | Stomatitis and skin better | Still abdominal distress, much better, skin improved, moderate diarrhea |
| | ..... Discharged ..... | | | | | |

two who served as controls had no significant illnesses (Table II). Within seven days after starting the deficient diet the four other men began to complain of various symptoms. The two who were deficient had abdominal distress, epigastric burning and headache. They passed a considerable amount of gas by eructations and as flatus. They complained of headache, dizziness, irritability and inability to concentrate. One man had aching joints, but there were no objective signs. He also had a mild cheilosis during the tenth week. Both

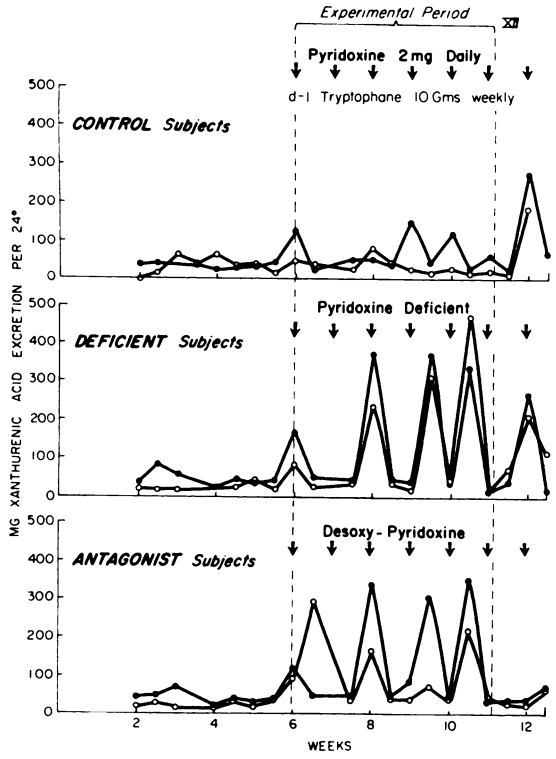


FIG. 1

men recovered promptly when pyridoxine was restored to their diet at the beginning of the eleventh week. The other two men, who were designated as antagonist subjects, became more severely ill. Their gastrointestinal symptoms were similar but in addition they had nausea, vomiting and diarrhea intermittently. One subject (No. 6) lost 10 pounds, but all the others maintained their weight. Epithelial changes were marked. Both men in the antagonist group had scaling of the skin of their face, scalp, arms and scrotum. Both had foul breath, severe gingivitis, soreness and discoloration of the tongue and dry cracked lips. Scrotal itching was particularly troublesome. The symptoms of headache, fatigue, dizziness and irritability were only moderate. None of the six men had significant alterations in temperature, pulse rate, blood pressure or respiratory rate. No objective neurologic changes could be demonstrated. After vitamins were restored to their diet and the antivitamin discontinued, one man recovered promptly and the other recovered gradually.

Routine examinations of the urine failed to demonstrate proteinuria, glycosuria or abnormal urinary sediment. The values for hemoglobin, hematocrit, erythrocyte sedimentation rate, white blood count and differential count remained normal in the two control subjects. All four men who were deficient became anemic. Their hemoglobin concentration decreased from an average of 15.5 to 12.5 gm., their red blood count from an average of 5.2 to 4.9 million per cu. mm. and their hematocrit from an average of 47 to 43 per cent. Neither leukocytosis nor lymphocytopenia was found.

Xanthurenic acid excretion increased in the four experimental subjects, but not in the two control subjects. After restoration of pyridoxine to their diet at the close of the study this abnormality was corrected (Fig. 1).

Antibody responses to tetanus and typhoid immunizations are shown in Table III. One of the two control subjects responded poorly. The two deficient men had low average responses and the two antagonist subjects responded slightly less well. Although the average response of all four deficient men was somewhat lower than usual, there was no absolute evidence of immunologic inhibition. A comparison of the responses of these men with those of men in previous series, however, led us to suspect that some inhibition had occurred (Fig. 2).

Measurement of the cholesterol content of the blood indicated that the inclusion of egg yolk in the formula of three of the men prevented the decrease in cholesterol concentration which was observed in the other three men whose formula contained corn oil as a source of lipid. Greenberg and Reinhart[5] reported that pyridoxine deficiency in monkeys resulted in a greater degree of hypercholesterolemia than in a control animal. In the present study there was no indication that pyridoxine deficiency changed the amount of cholesterol in their blood.

## COMMENTS

Pyridoxine deficiency can be induced in two ways: by depriving men of the vitamin or by giving large doses of the antagonist, desoxypyridoxine. In the present study the combined

TABLE III
Antibody Responses

| No. | Subject | Week No. | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 13 |
| | | *Tetanus Antibodies* | | | | | | |
| 1 | Control | 4.0 | 3.6 | 3.8 | 3.4 | 8.4 | 21.8 | 29.6 |
| 2 | Control | 0.01 | 0.01 | 0.01 | 0.01 | 0.4 | 1.4 | 2.0 |
| 3 | Deficient | 0.01 | 0.01 | 0.01 | 0.01 | 5.6 | 12.8 | 4.6 |
| 4 | Deficient | 3.2 | 3.0 | 3.4 | 2.0 | 5.4 | 8.0 | 4.4 |
| 5 | Antagonist | 0.2 | 0.6 | 0.6 | 0.4 | 5.6 | 12.0 | 4.8 |
| 6 | Antagonist | 3.0 | 2.8 | 3.0 | 3.0 | 4.0 | 4.2 | 1.6 |
| | | *Typhoid O Antibodies* | | | | | | |
| 1 | Control | 80 | 80 | 80 | 40 | 640 | 640 | 640 |
| 2 | Control | 40 | 40 | 40 | 10 | 640 | 640 | 640 |
| 3 | Deficient | 40 | 40 | 40 | 40 | 320 | 640 | 320 |
| 4 | Deficient | 80 | 80 | 80 | 20 | 640 | 320 | 320 |
| 5 | Antagonist | 5 | 5 | 5 | 5 | 1,280 | 320 | 320 |
| 6 | Antagonist | 80 | 80 | 80 | 20 | 80 | 80 | 80 |
| | | *Typhoid H Antibodies* | | | | | | |
| 1 | Control | 20 | 20 | 20 | 20 | 2,560 | 1,280 | 1,280 |
| 2 | Control | 20 | 20 | 20 | 10 | 1,280 | 640 | 640 |
| 3 | Deficient | 20 | 20 | 20 | 10 | 1,280 | 640 | 640 |
| 4 | Deficient | 40 | 20 | 20 | 20 | 640 | 640 | 320 |
| 5 | Antagonist | 20 | 20 | 20 | 5 | 160 | 320 | 640 |
| 6 | Antagonist | 80 | 40 | 40 | 20 | 160 | 160 | 80 |

regimen resulted in more severe symptoms and signs of illness. The epithelial changes which occur in pyridoxine deficiency have been likened to seborrheic dermatitis.[6] In the present study the two subjects who received the antagonist had such severe inflammatory changes, scaling, itching and desquamation as to suggest exfoliative dermatitis. They also had severe inflammatory changes of their gums, lips and tongue. We noted that men who had their own teeth had much more severe gingival reaction than those who wore dentures. Inclusion of egg yolk as a source of fats and cholesterol in the diet of one man from each pair did not influence the cutaneous abnormalities.

Pyridoxine deficiency has been reported to cause anemia in swine[7] and in man.[8] In experimental human deficiency, anemia has not been a prominent feature. In one of two infants deprived of pyridoxine severe microcytic hypochromic anemia did develop but this was corrected by the restoration of pyridoxine to the diet.[9] In animals, pyridoxine deficiency has been reported to result in polymorphonuclear leukocytosis and in a rather marked lymphopenia.[10] This effect was reported by Vilter and his group (*loc. cit.*) to be scarcely detectable in man. In the subjects in the present study a modest decrease did develop in hemoglobin concentration, erythrocyte count and hematocrit, but there was no granulocytosis or lymphopenia. The anemia was not due to blood lost by venapuncture since there was no change in the control subjects.

In a study such as this, gastrointestinal symptoms are difficult to evaluate. The recorded symptoms were nondescript and in many ways resembled those of men who were deficient in pantothenic acid.[11] The lack of

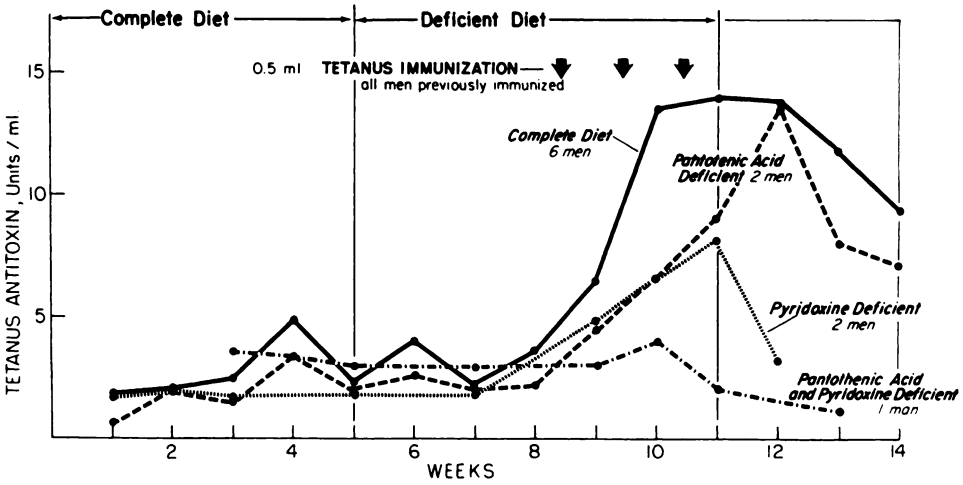


FIG. 2

symptoms in the control subjects indicates that the formula was not responsible.

Neurologic symptoms have been described in many instances of pyridoxine deficiency. Convulsive seizures occurred in infants fed a pyridoxine deficient diet.[12] Large doses of the antagonist desoxypyridoxine will produce convulsive disorders in animals.[13] In the present study the subjects showed no evidence of excitation aside from the moderate irrascibility which any ill patient may have. There was no intensification of the deep tendon reflexes and no tremor or incoordination. The headaches and slight dizziness may have been part of their general illness.

Pyridoxine is essential for several phases of protein metabolism, yet the exact roles which it plays are not clearly understood. Such vital functions as decarboxylation, transamination and desulfhydrase activity are dependent upon this vitamin.[14–16] Little is known about the requirements for synthesis of gamma globulin or of antibodies. Animal experiments indicate that pyridoxine is essential for the formation of antibodies and hence presumably of gamma globulin (*loc. cit.*). Our study furnishes little evidence of a disturbance in protein metabolism although it suggests that pyridoxine deficiency in man may hamper antibody formation to some degree.

Xanthurenic acid excretion in the urine is well established as an index of pyridoxine deficiency. This has been well summarized by Price and his colleagues.[17] All four deficient subjects had a significant increase in the amount of xanthurenic acid excreted in their urine. This finding, coupled with their clinical illness and the rather prompt response to the restoration of vitamins to their diet, indicates that the illness was produced by a deficiency of pyridoxine.

## SUMMARY

Six men were fed a semi-synthetic formula adequate in all known nutrients except pyridoxine. Two men served as control subjects and received adequate amounts of pyridoxine. The remaining four men were deficient in pyridoxine. Two of them received, in addition, the anti-vitamin desoxypyridoxine. All six men were immunized with tetanus and typhoid antigens. The four deficient subjects became ill while the two control subjects remained well. The most notable features of this illness were gastrointestinal disturbances and cutaneous changes. The formation of antibodies against tetanus and typhoid was only slightly impaired in the deficient subjects.

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