CLINICAL AND BIOCHEMICAL STUDIES OF PYRIDOXINE DEFICIENCY IN PATIENTS WITH NEOPLASTIC DISEASES

S. D. GAILANI, MB,* J. F. HOLLAND, MD,*
A. NUSSBAUM, BS,* AND K. B. OLSON, MD†

Seventeen patients with advanced neoplastic diseases were fed a formula diet deficient in vitamin B_6 for 10 to 80 days. In addition, nine of the patients received the B_6 antagonist, 4-deoxypyridoxine (4-DOP), for periods ranging from 6 to 46 days. No definite antitumor effect could be observed in any of the patients studied despite ample biochemical and clinical evidence of vitamin B_6 depletion. The study confirmed previous reported experiences in man and many other animal species that 4-DOP accentuates some of the manifestations of vitamin-B_6 deficiency, particularly neurologic and dermatologic side effects. The lymphopenic effect of 4-DOP was demonstrated only in patients with normal bone marrow. In three of four patients with lymphoproliferative disease the peripheral lymphocyte count rose coincidentally with 4-DOP administration while in the fourth the rise developed immediately after discontinuation of 4-DOP and at the peak of toxicity. The study showed evidence of progressive tissue depletion when the patient was fed the deficient diet alone. Addition of 4-DOP to the deficient diet led to the transient reappearance of measurable pyridoxal phosphate in leukocytes and pyridoxic acid in the urine. This and the slight increase in reticulocyte counts during administration of 4-DOP raise the possibility that vitamin B_6 was mobilized from certain tissue depots by the 4-DOP and was thus available for oxidation to pyridoxic acid and for use by the less depressed of the enzymes dependent upon vitamin B_6.

VITAMIN-B_6 DEFICIENCY INDUCED BY A DIET deficient in vitamin B_6 alone[1, 8] or in combination with one of the vitamin-B_6 antagonists[19, 20] produces regression or growth retardation of many experimental murine tumors. The use of the antagonist alone, however, usually has been ineffective.[2] The vitamin B_6 antagonist, 4-deoxypyridoxine (4-DOP), has been investigated as a therapeutic agent for cancer in man. It was of no value when given with a diet low in vitamin B_6 for short periods[5] and of limited use in acute lymphoblastic leukemia in adults when given with a regular diet for more prolonged periods.[24]

The recent availability of a purified vitamin-deficient diet, suitable for prolonged human consumption, made it possible to reinvestigate the effects of vitamin-B_6 deficiency on human neoplastic diseases under rigorously controlled dietary conditions. The clinical, biochemical and other laboratory observations made on 17 patients with various neoplastic diseases who took the diet deficient in vitamin B_6 with or without 4-deoxypyridoxine supplementation is the subject of this communication.

MATERIALS AND METHODS

Seventeen patients were studied—14 in Roswell Park Memorial Institute, two in the Albany Medical Center and one in the United

From the Departments of Medicine, Roswell Park Memorial Institute, Buffalo, N.Y.,* and Albany Medical College of Union University, Albany, N.Y.†

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Address for reprints: S. D. Gailani, MB, Department of Medicine A, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, N.Y. 14203.

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States Public Health Service Hospital in Boston. Each of the 17 patients studied had a definitely diagnosed neoplastic disease with measurable parameters for proper evaluation of the effects of treatment. Sixteen patients had an estimated survival of longer than 3 months at the onset of the study. The treatment was carried out in metabolic wards. The patients gave informed voluntary consent, and each agreed to a tentative period of hospitalization of not less than 3 months.

The patients were chosen from among hospital patients who became refractory to other forms of treatment, when no known effective treatment was available, or when it was believed that postponement of other forms of noncurative treatment would not be injurious to the patient. Special effort was made to include some patients with lymphoproliferative diseases because of the known lymphopenic effect of vitamin-B_6 deficiency in man and many animal species.

During a period of observation averaging 7 days, the patients were given a regular hospital diet or formula (for three tube-fed patients) and the daily caloric intake was calculated from tables by a research dietitian. Baseline studies were performed including hemoglobin concentration, hematocrit, red cell and reticulocyte counts, white cell and differential counts, platelet counts, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Bone marrow aspiration was performed on 12 patients.

The following clinical laboratory tests were performed: blood urea nitrogen, serum uric acid, alkaline phosphatase, bilirubin, electrolytes, total protein and albumin, total and free cholesterol, glutamic oxalacetic transaminase (SGOT) and serum iron. Prothrombin time and cephalin flocculation tests were performed on three. Tests to assess vitamin-B_6 status were performed on the patients studied in Roswell Park Memorial Institute, including measurement of pyridoxal phosphate[3] in isolated leukocytes of 12 patients, in liver of seven (needle biopsy), and in surgical biopsies of tumors of four patients. Twenty-four-hour urinary excretion of pyridoxic acid[14] was measured in 12 patients and xanthurenic acid excretion[16] after 2 or 5 Gm of L-Tryptophan load was done in seven. Glutamic oxalacetic transaminase measurement in isolated leukecytes[9] was performed in two patients.

After the caloric intake of each patient was estimated and the baseline studies were performed, the planned treatment was started. Patients consumed a liquid formula diet deficient in vitamins except vitamin E. All other foodstuffs except tea and coffee with sugar or saccharine were excluded. The deficient diet is a semisynthetic formula diet prepared by the Mead Johnson Company. It was supplied in powder form in sealed cans, each can containing 147 Gm of the diet formula (700 calories). In addition to protein, fats and carbohydrates, the formula contained all the essential minerals in sufficient quantities (Table 1).

The contents of each can were blended in 400 cc of tap water on the day it was served and stored in a refrigerator until used. The patients ordinarily were given 6 feedings between 8 AM to 10 PM to a total caloric level equal to their baseline consumption.

Vitamins other than B_6 were supplied daily in adequate amounts by two chewable multivitamin tablets specially prepared for the study by Mead Johnson Company (Table 1). The last six patients were given 150 to 200 mg of choline hydrochloride in a single daily dose when re-analysis of the formula revealed a total lack of choline. The analysis for choline content of the diet was prompted by the development of fatty changes in the livers of

TABLE 1. Contents of Each Can of Vitamin-deficient Diet and Daily Vitamin Supplementation

Contents	Amount per can	Supplementation
Net weight	147 Gm	
Caloric equivalent	700 calories	
Dextrose and sucrose	72.5 Gm	
Corn oil	30 Gm	
Casein	35 Gm	
Calcium	0.5 Gm	
Phosphorus	0.5 Gm	
Iron	7.5 mg	
Iodine	0.075 mg	
Sodium	0.5 Gm	
Potassium	1.0 Gm	
Magnesium	0.15 Gm	
Copper	1.0 mg	
Manganese	2.0 mg	
Zinc	5.0 mg	
Vitamin A	0	8000 units
Vitamin D	0	800 units
Thiamine	0.007 mg	2.4 mg
Riboflavin	0.004 mg	3.0 mg
Nicotinamide	0.2 mg	30.0 mg
Pyridoxine	0.02 mg	—
Calcium pantothenate	<0.06 mg	10 mg
Vitamin B_{12}	0.03μg	6μg
Folic acid	0.002 mg	0.6 mg
Ascorbic acid	0.45 mg	150 mg
α Tocopherol	10 units	—
Biotin	—	80μg

TABLE 2.

Case no.	Diagnosis	Age (yr)	Sex	Days on deficient formula	Days on thrice boiled diet	Days and duration of 4-DOP	Reason for discontinuation	Clinical side effects
1	Small cell lymphosarcoma	35	M	10	—	10*	Rapidly progressive disease	None
2	Reticulum cell sarcoma	59	M	11	—	11*	Death unrelated to the deficiency study	None
3	Metastatic carcinoma of breast	78	F	24	6	—	Marked loss of appetite and mental depression	Weakness
4	Metastatic pancreatic carcinoma	52	M	24	—	—	Progressive disease	None
5	Multiple myeloma	43	F	35	—	—	Anemia, poor general condition	None
6	Cancer of esophagus	38	F	38	—	—	Poor general condition, severe anemia	Twitchings in right upper extremity, EEG changes in left temporal region
7	Multiple myeloma	65	F	40	—	—	Acute abdomen (possibly cholecystitis)	None
8	Small cell lymphosarcoma	53	M	51	—	13† (38–51)	Progressive disease	Weakness, paresthesia, mild dermatitis
9	Chronic lymphocytic leukemia	57	M	51	14	14* (52–65)	Anorexia, paresthesia	Anorexia, dermatitis, paresthesia
10	Cancer of larynx	53	M	53	—	25‡ (18–36), (47–53)	Paresthesia	Paresthesia, dermatitis
11	Cancer of pharynx	57	M	55	—	—	—	Weakness, mild scaliness of skin
12	Chronic lymphocytic leukemia	72	F	60	—	46‡ (11–57)	—	Generalized dermatitis, paresthesia
13	Bronchial adenoma and carcinoid syndrome	69	F	61	—	28* (3–31)	Anorexia	Mild dermatitis, paresthesia
14	Cancer of breast	63	F	61	14	14*	Anorexia, paresthesia	Anorexia, dermatitis, paresthesia
15	Hodgkin's disease	22	M	65	—	6†	Paresthesia	Paresthesia, dermatitis
16	Cancer of larynx	62	M	73	—	—	—	Accentuation of pre-existing seborrhea of scalp
17	Bronchogenic carcinoma	56	M	80	—	—	Progressive disease	

* 4-DOP batch NSC 3063.
† 4-DOP batch NSC 3063 after chromatographic purification.
‡ B_6-free 4-DOP.

some of the early patients receiving the diet.

To extend the period of vitamin-B_6 depletion, patients 3, 9 and 14 (Table 2) were given a whole diet low in vitamin B_6 in the period immediately after discontinuation of the vitamin-deficient formula. This diet comprised foodstuffs known for their low B_6 content. The food was boiled three times in large quantities of water in a manner similar to that advocated by Herbert for the preparation of a low folate diet.[6] This approach was discontinued because of the unpalatability of the diet and the difficulty of sustaining vitamin-B_6 deficiency despite it.

Nine of the 17 patients also received the vitamin-B_6 antagonist, 4-DOP, in doses of 0.5 to 1.5 mg/kg of body weight per day for variable periods during the vitamin-B_6 depletion. The compound was supplied in powder form and was made into capsules and given in three equally divided doses daily. Five patients, 1, 2, 9, 13 and 14 (Table 2), received 4-DOP from batch NSC 3063.

While the study was in progress, Dr. Alex Bloch of the Department of Experimental Therapeutics at Roswell Park demonstrated the presence of pyridoxine (0.05%) in the 4-DOP we were using. The separation of these

two compounds was accomplished chromatographically on Whatman 3 MM paper sheets. The mixture was applied to the paper in a band, and a second band consisting of a saturated borate solution was placed immediately in front of it. N-propanal 80% and aqueous formic acid 20% were used as the solvent. Two ultraviolet absorbing bands were located. One corresponded to the 4-DOP, capable of inhibiting the growth of Saccharomyces carlsbergensis. The other, with a migration corresponding to pyridoxine, was capable of substituting for the vitamin in supporting the growth of the test organism.

A purification and separation procedure was accomplished using the chromatographic technique for batch lots. The 4-DOP zone was excised and eluted and the product was crystallized. This purified preparation was given to patients 8 and 15 (Table 2). Since prior 4-DOP had always been made by the same synthetic route starting with pyridoxine, it is likely that all 4-DOP has had some vitamin-B_6 activity and all prior biological investigation should be reinterpreted accordingly. A new batch of 4-DOP was made by Merck, Sharp and Dohme using a new synthetic route[22] which did not start with pyridoxine. This uncontaminated 4-DOP was given to patients 10 and 12 (Table 2).

The diagnoses, age and sex distribution of the patients are presented in Table 2. They were given the vitamin deficient diet for 10 to 80 days. In addition, patients 3, 9 and 14 received the thrice boiled diet low in vitamin B_6 for 14, 6 and 14 days in the period immediately after the termination of the vitamin-deficient formula diet (Table 2). Nine of the patients were given 4-DOP for 6 to 46 days during the period of vitamin-B_6 deprivation. Patients 9 and 14 (Table 2) received the 4-DOP immediately after the discontinuation of the formula diet but with the thrice-boiled diet low in vitamin B_6. Four patients received the 4-DOP during the early part of the deficient diet while three others received it towards the end of the study.

RESULTS

Tests to Assess Vitamin B_6 Status: There are three tests to assess status of vitamin B_6:

Pyridoxal phosphate (PLP) measurement in isolated leukocytes and other tissues—PLP measurement in isolated leukocytes was performed on 12 patients on the average of once a week. There was a gradual and usually progressive drop in the level of PLP in the isolated leukocytes to unobtainable levels in all patients receiving the deficient diet alone (Fig. 1). When 4-DOP was added to the deficient diet, leukocyte PLP increased or reappeared. Moreover, there was a marked fluctuation in the level of leukocyte PLP during the period of 4-DOP administration (Fig. 4). These changes occurred with both the original batch of 4-DOP and the new preparation which was free of vitamin-B_6 contamination. There was a progressive decrease in the level of liver PLP in six of the seven patients studied (Fig. 2). There was no apparent difference in the rate of hepatic depletion of PLP between the four patients who received the deficient diet alone and those who received both the deficient diet and the 4-DOP. Repeated tumor biopsies were performed on four patients while they were receiving the deficient diet alone; assay for PLP in the tumors showed a definite decline in all four (Fig. 3). In the one patient who had a muscle biopsy, PLP content of muscle declined from a baseline of 4.1 μg/Gm to 2.6 μg/Gm on day 30 (Fig. 4).

Pyridoxic acid (PIC) excretion—There was a rapid progressive decline in the pyridoxic-acid excretion in the urine when patients were fed the deficient diet alone (Fig. 5). 4-DOP supplementation resulted in a reappearance of pyridoxic acid in the urine. The amount excreted under these circumstances was usually modest (less than 400 μg/24-hour urine).

Xanthurenic acid excretion—Xanthurenic-acid excretion after tryptophan load increased during the depletion periods in all seven patients on whom it was performed (Fig. 6). The test was repeated in two patients in the poststudy period at a time when they were receiving 20 mg of pyridoxine a day; the xanthurenic-acid excretion had dropped to 8 and 10 mg/24 hours.

Serum and leukocyte glutamic oxalacetic transaminase: Only one of 11 patients showed a progressive decline of SGOT activity during the period of feeding the deficient diet alone. On the other hand, a definite and usually

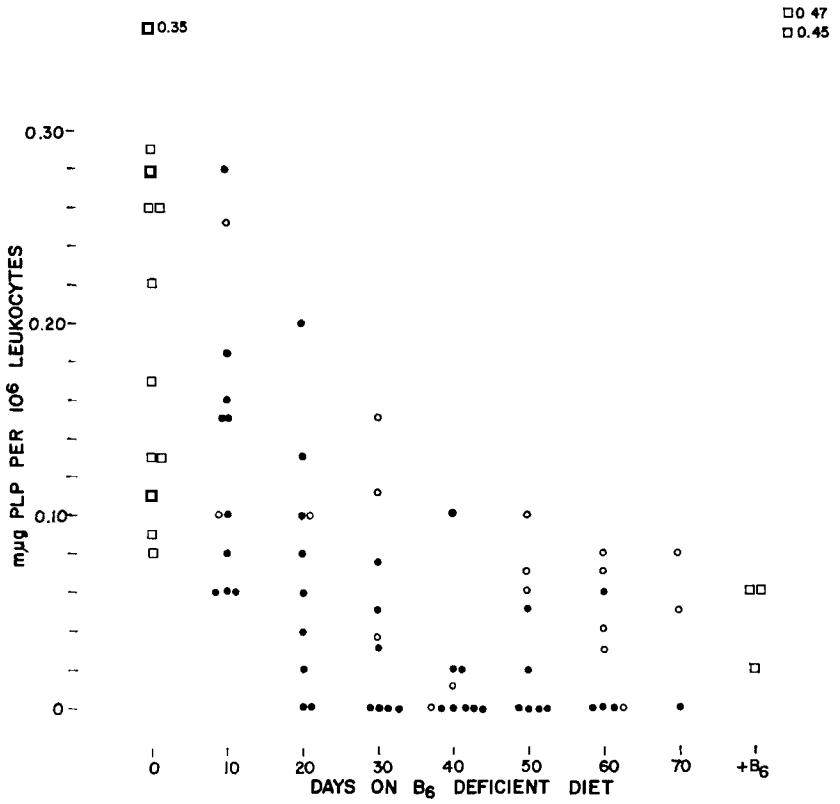

FIG. 1. Pyridoxal phosphate (PLP) in isolated leukocytes (mean of measurements in preceding 10 days in individual patients) during the period of feeding the B_6-deficient diet alone (•) and when 4-DOP was added to the B_6-deficient diet (0).

progressive depression of SGOT occurred in all patients during 4-DOP administration. SGOT activity was recovered rapidly after vitamin-B_6 supplementation (Fig. 7). Leukocyte GOT activity roughly paralleled that of the serum.

Hematologic changes: Twelve of the 17 patients had a decline in hemoglobin concentration. A corresponding decline in hematocrit and red cell count was observed when these tests were done. The reduction in hemoglobin concentration was slight to moderate

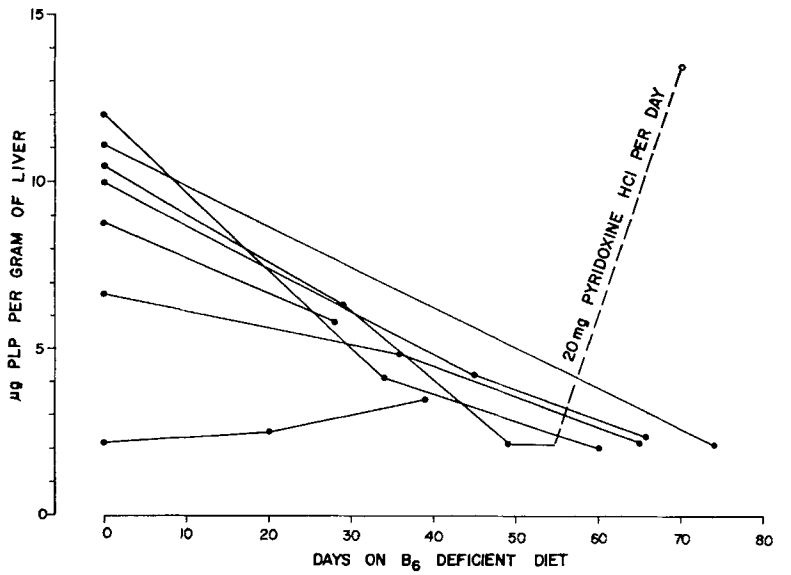

FIG. 2. Hepatic pyridoxal phosphate levels before and during the period of feeding the B_6-deficient diet in seven patients and the effect of adding pyridoxine after the B_6-depletion period in one patient.

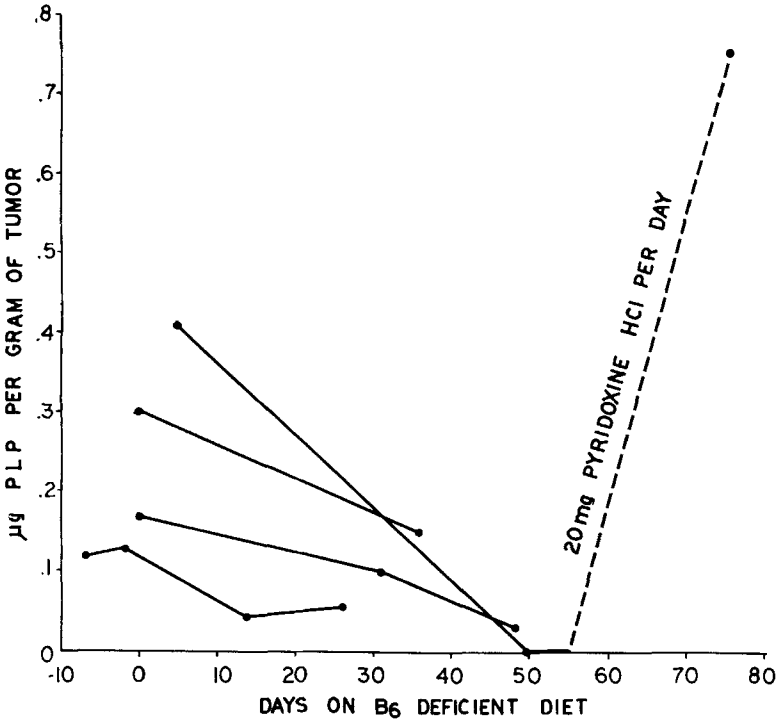

FIG. 3. Tumor pyridoxal phosphate levels before and during the period of feeding the B_6-deficient diet and the effect of adding pyridoxine after the B_6-depletion period in one patient.

(0.6 to 1.9 Gm/100 ml) in seven patients. When the hemoglobin drop was more marked, additional factors other than vitamin-B_6 deficiency seemed to have played a role. These included marrow infiltration by the neoplastic process, previous radio-or chemotherapy, bleeding, infection and hypothroidism. Another contributing factor was the 50 to 100 ml of blood drawn each week from all patients.

Definite decrease in the reticulocyte counts was observed in four of nine patients studied while taking the deficient diet alone; the other five showed no change. Five of seven

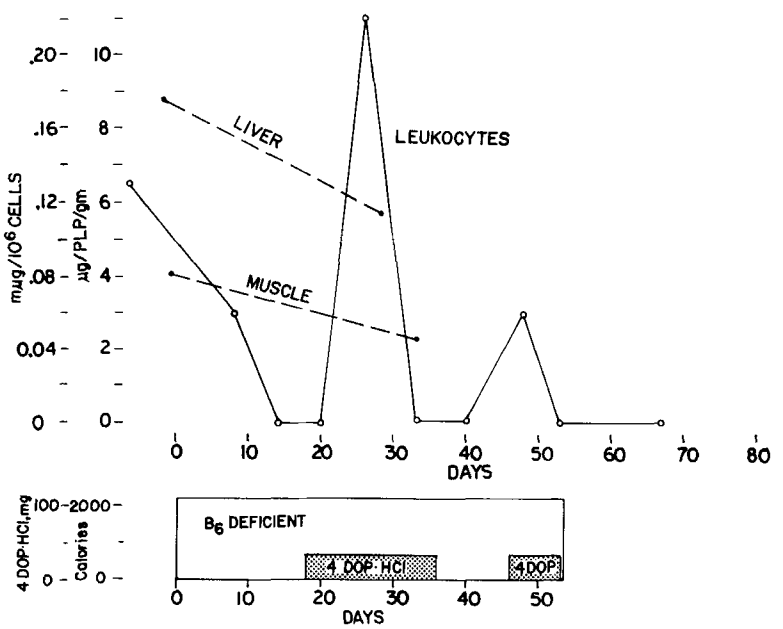

FIG. 4. Measurements of pyridoxal phosphate in liver, muscle and isolated leukocytes (patient 10) during the feeding of the B_6-deficient diet with two periods of 4-DOP supplementation.

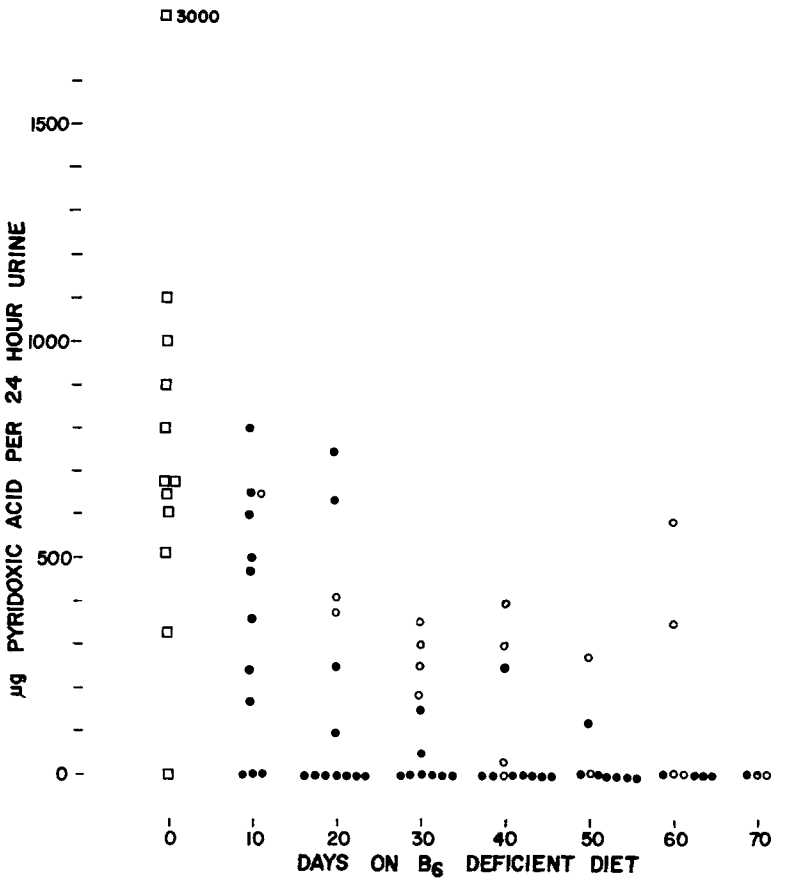

FIG. 5. Twenty-four hour urinary excretion of pyridoxic acid (mean of measurements in the preceding 10 days in individual patients) during the period of feeding the B_6-deficient diet alone (•) and when the deficient diet was supplemented by 4-DOP (0).

patients had an increase in the reticulocyte count while taking 4-DOP in addition to the diet deficient in vitamin B_6 and only one patient had a decrease (Fig. 8). These increases in reticulocyte count occurred without evidence of bleeding, hemolysis or a definite change in the hemoglobin concentration. No definite changes were found in the red cell indices, marrow aspiration studies, platelet counts or serum iron levels.

Changes in white cell counts were confined to the lymphocytes and occurred only when the deficient diet was supplemented by 4-DOP. Definite lymphopenia developed in four patients with nonlymphoid neoplasms and presumably normal marrow function (Fig. 9), when the diet deficient in vitamin B_6 was supplemented with 4-DOP for 6 to 28 days. Of four patients with lymphoproliferative disease, however, three developed a rise in peripheral lymphocyte count temporally related to 4-DOP administration (Fig. 9).

One of these patients had chronic lymphocytic leukemia and two had lymphosarcoma which eventually infiltrated the marrow and developed a peripheral blood picture undistinguishable from chronic lymphocytic leukemia. One of these patients was a 58-year-old man with a 9-year history of small cell lymphosarcoma who previously had been treated with x-ray irradiation and nitrogen mustard but had no treatment in the preceding 2½ years. There had been a gradual progression of the disease manifested by increasing hepatosplenomegaly, lymphadenopathy, infiltration of the marrow by lymphocytes up to 88% and increase in the percentage of lymphocytes in the peripheral blood count up to 65%. The patient was fed the diet deficient in vitamin B_6 for 51 days, the last 13 of which the diet was supplemented by 4-DOP. A definite increase in the lymphocyte count occurred coincidental with 4-DOP administration (Fig. 10) but without change in lymphadenopathy or organomegaly. A bone marrow on day 50 contained 94% lymphocytes. A similar paradoxical rise of lymphocytes during 4-DOP administration was observed in the second and third patients.

Because of the possibility that the disease in

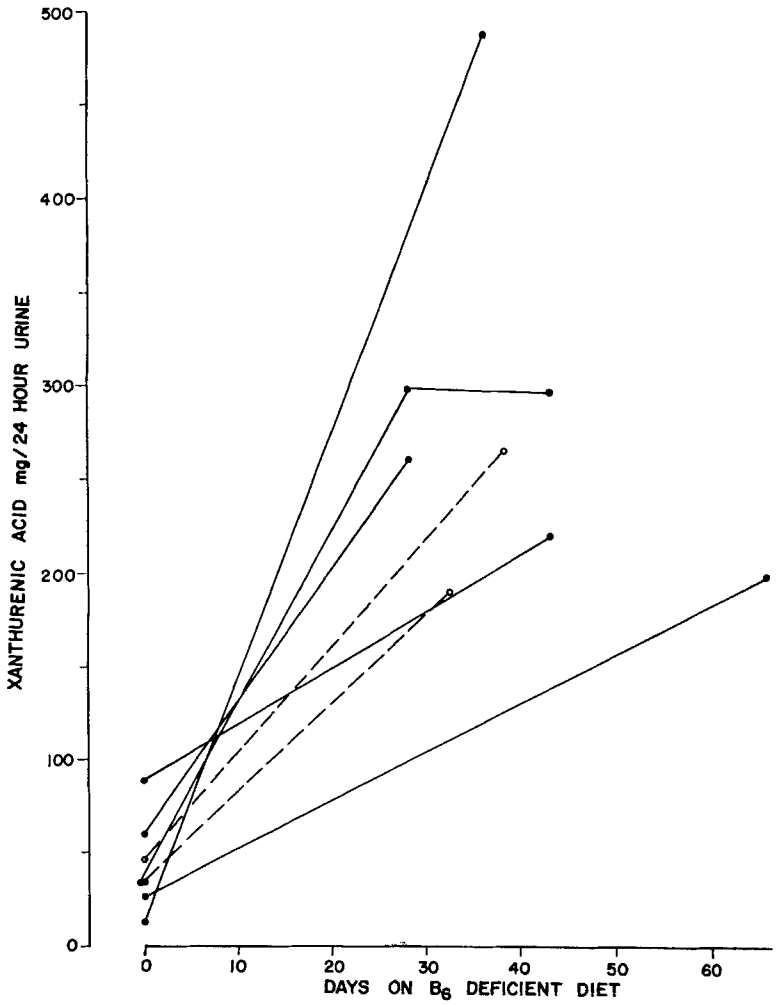

FIG. 6. Urinary xanthurenic acid excretion after 2 Gm (interrupted line) or 5 Gm (solid line) L-tryptophan load before and during B_6 depletion.

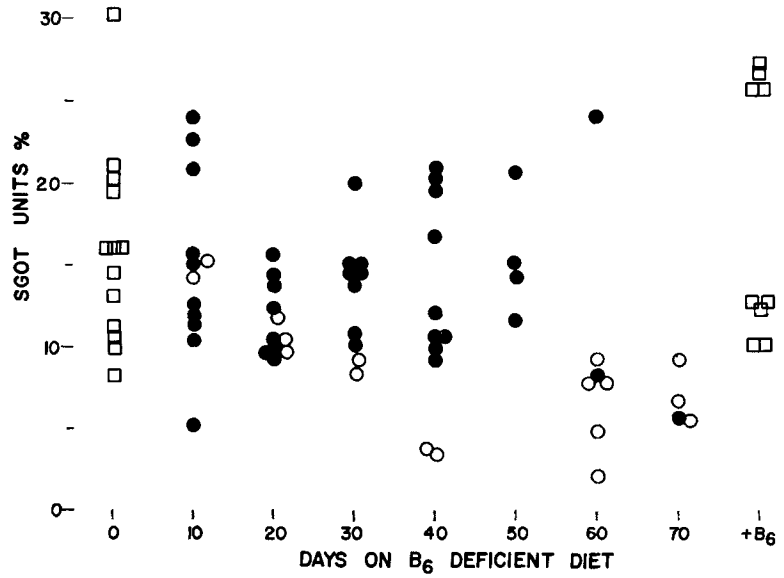

FIG. 7. SGOT (mean of measurements in the preceding 10 days in individual patients) during the period of feeding the B_6-deficient diet alone (•) and when the deficient diet was supplemented by 4-DOP (0).

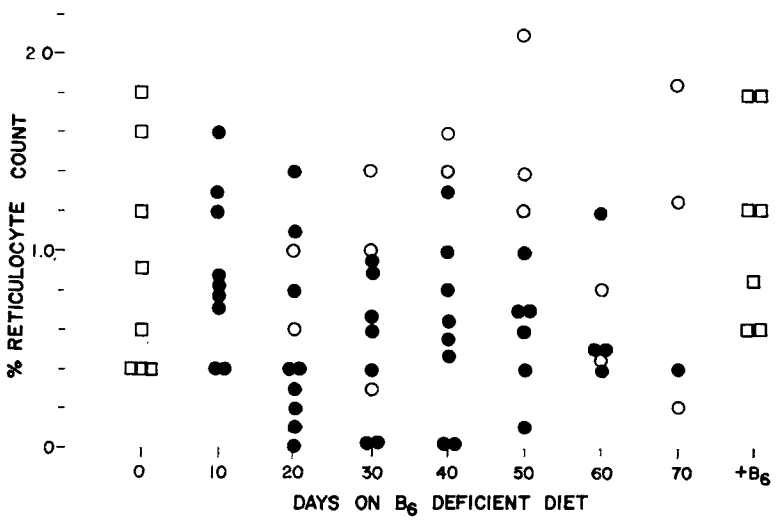

FIG. 8. Reticulocyte counts (mean of counts in the preceding 10 days in individual patients) during the period of feeding the B_6-deficient diet alone (•) and when the deficient diet was supplemented by 4-DOP (0).

these three patients was in an accelerated phase and the effect of 4-DOP on raising the lymphocyte count was only apparent, we tried the treatment on a patient with stable chronic lymphocytic leukemia. She was asymptomatic and her physical examination was normal. During a 5-month period of observation her white cell count ranged between 125,000 to

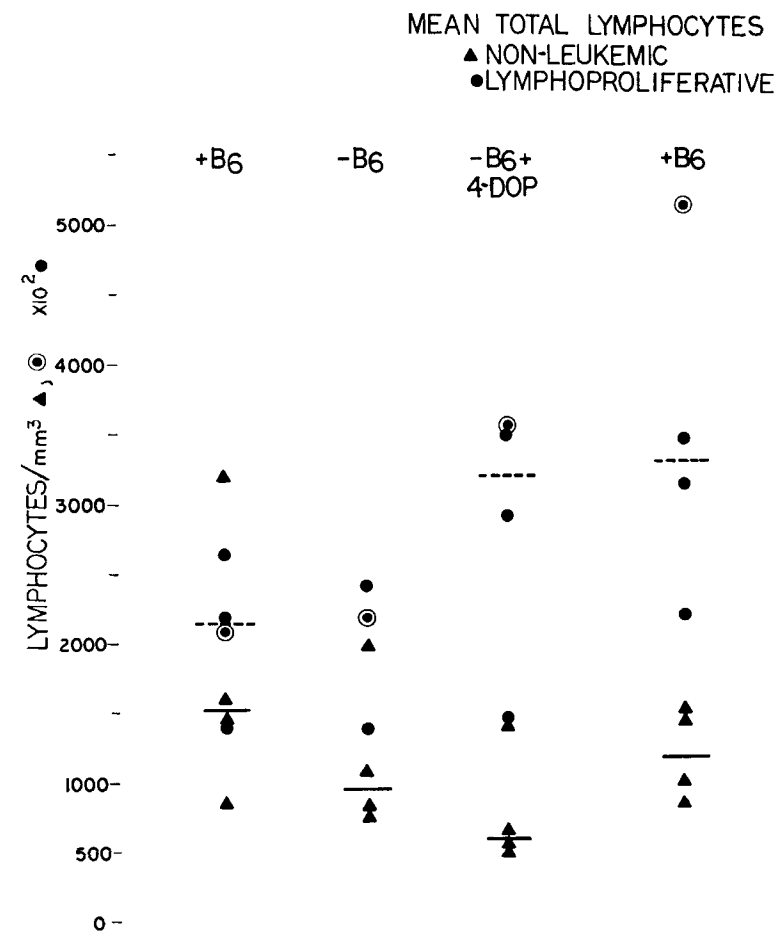

FIG. 9. Mean of measurements of peripheral lymphocyte counts during the period of feeding the B_6-deficient diet alone and when the diet was supplemented by 4-DOP as compared to peiods before and after B_6-depletion periods in patients with normal marrows and in those with lymphoproliferative diseases.

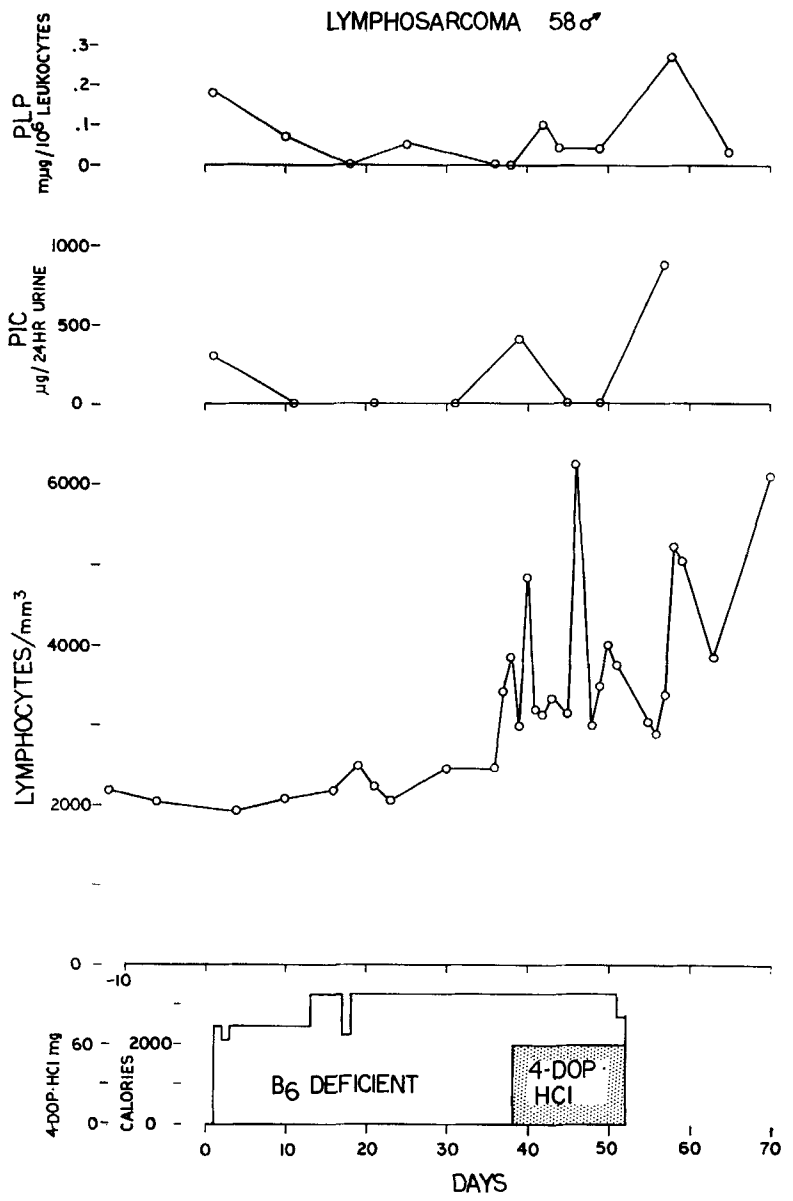

FIG. 10. Peripheral lymphocyte counts, pyridoxal phosphate in isolated leukocytes and urinary excretion of pyridoxic acid (PIC) during the period of feeding the B_6-deficient diet alone and when the deficient diet was supplemented by 4-DOP in patient 8 with lymphosarcoma.

140,000/cumm with 96 to 99% small lymphocytes. She was given the diet deficient in vitamin B_6 for 60 days during which the diet was supplemented with 4-DOP for the last 46 days. No change in the lymphocyte count was observed during this 60-day period and the patient was discharged on a regular diet without vitamin-B_6 supplementation. A generalized scaly erythematous rash developed three days after her discharge with severe paresthesia in the hands and feet. Her white count 21 days after discharge was 200,000/cumm and it remained at the new plateau for one year of follow-up.

Neurologic changes: Paresthesia developed in seven of the nine patients who received 4-DOP. They complained of a prickling sensation in the hands and feet. The paresthesia cleared completely within 3 weeks in five patients but in the other two it persisted in slowly decreasing severity for more than 6 months. There were no objective sensory or motor changes in conjunction with the paresthesias. Mild paresthesia of the hands developed in only one patient when fed the deficient diet alone. The paresthesia developed on day 50 and cleared within a week after vitamin-B_6 supplementation.

Involuntary twitching of the right hand and forearm appeared in case 6 on day 36 of the dietary depletion period; no 4-DOP had been given. The electroencephalogram (EEG) taken on that day showed slowing and increased voltage in the left temporal region. The twitching and EEG changes cleared after vitamin-B_6 supplementation. An autopsy performed a month after the discontinuation of the study did not show metastases in the brain. Two other patients developed generalized diffuse slowing of the EEG but without any motor phenomena; both patients were old (67 and 78 years).

Changes in skin and mucous membrane: Only two patients developed skin changes during the time they were taking the deficient diet alone. A more definite and more severe dermatitis occurred in seven of the nine patients who received the deficient diet and 4-DOP. The longer the patient had been taking the deficient diet, the sooner the dermatitis appeared when 4-DOP was administered. In one patient dermatitis appeared 3 days after reinstitution of a regular diet following 46 days of 4-DOP. The most common locations of the dermatitis were the nasolabial folds, other skin folds and forehead; one patient developed generalized dermatitis. The lesions consisted of erythematous scaly rash with ill-defined borders accompanied by moderate itching and irritation (seborrhea sicca-like dermatitis).[23] The lesions cleared within 7 to 21 days after vitamin-B_6 supplementation.

Slight conjunctival infection and blepharitis were found in four of the nine patients receiving 4-DOP. Seven of the nine patients receiving 4-DOP developed a burning sensation of the mouth which many described as a feeling of having their mouth scalded by a hot drink. Only a mild infection of the oral mucous membrane was noted in these patients. Mild cracking of the lips and angular stomatitis also were observed. The lingual papillae became slightly denuded in one patient.

Liver biopsy and liver function tests: The first three patients who had repeated biopsies of the liver developed fatty infiltration of the liver. This change was evident when the biopsy was repeated 20 to 28 days after the beginning of the study and persisted throughout the period of feeding the deficient formula diet. Addition of 20 mg of pyridoxine hydrochloride daily to the deficient diet for 2 weeks failed to clear the fatty infiltration in one of the patients. Analysis of the diet revealed a total lack of choline. Another two patients were fed the choline-deficient formula with the intention of demonstrating the therapeutic action of choline but no evidence of fatty changes was found on biopsies in these two patients. Nevertheless, choline was added to the dietary regimen thereafter and fatty liver was not encountered in the last two patients who had serial biopsies of the liver.

A moderate rise in serum alkaline phosphatase (by 5 to 12 KA units) was observed in six patients (only one of these patients had metastases to liver and none had metastases to bone). The rise was usually apparent after day 45 of feeding the deficient formula. In the three patients who had serial alkaline phosphatase measurements in the poststudy period the enzyme activity reverted to prestudy levels within 10 to 30 days after the return to a regular diet. On the other hand, supplementation of the diet with vitamin B_6 failed to reverse the rise in alkaline phosphatase in one patient and only partially corrected it in another. No rise in alkaline phosphatase was observed in the two patients who had choline added to the diet throughout the study periods of 55 and 60 days.

A drop in serum cholesterol ranging from 25 to 50% of the prestudy level occurred in all patients. The drop involved both the free and esterified portions without disturbing their ratio. The addition of choline to the formula of three patients and of pyridoxine to that of another did not change the cholesterol levels. A quick increase in the cholesterol levels occurred upon feeding either a regular diet or the thrice-boiled diet, reaching pretreatment levels within 1 to 2 weeks.

There was some tendency toward lowering of the total serum protein in the patients who took the formula diet for more than 40 days. The drop could be accounted for mostly by a decline of 0.3 to 0.9 Gm/100 ml in the serum albumin.

Blood Urea Nitrogen: The blood urea nitrogen (BUN) rose appreciably in two patients. One of the patients, who had an advanced and rapidly progressive preterminal lymphosarcoma with uric acid concentration of 6.7 mg/100 ml, had an increase in BUN from 18 to 56 mg/100 ml during a 10-day period while receiving the formula diet and 4-DOP. The BUN fell to 30 mg/100 ml 4 days after the discontinuation of the regimen. The other patient had bronchial carcinoid and

sustained a progressive rise in BUN from 11 to 49 mg/100 ml by day 55. This rise took place despite symptomatic improvement. There was no urinary-tract infection, crystalluria or hyperuricemia. Serum creatinine on day 55 was 1.1 mg/100 ml; no baseline serum creatinine was available. Pyridoxine (2.4 mg/day) was given to the patient for 10 days between day 62 and 72 while she continued on the same diet formula but the BUN continued between 42 and 49 mg/100 ml. After 2 weeks on a regular diet the patient's BUN had fallen to 16 mg/100 ml. Her food intake, and hence protein intake, was constant most of the time on the liquid diet.

Urinary changes: Oxalate crystals appeared in the urinary sediments in three patients. No other consistent urinary abnormality was observed in the other patients.

Appetite, weight and caloric intake: Two patients gained weight despite the pyridoxine-deficiency treatment periods of 28 and 40 days.

Four patients maintained their weight for 11 to 60-day study periods. None of the three tube fed patients developed anorexia, nausea or vomiting during the 38 to 73-day study periods.

Eight patients lost weight. In four patients this was unrelated to the diet or to the deficiency state (increased intake of formula prevented further loss in three; rapidly progressive cancer was evident in the fourth). In the remaining four patients who lost weight, this appeared when anorexia, followed soon by nausea and occasional vomiting, developed after 20 to 46 days of ingesting the formula diet. Only one of these patients was taking 4-DOP at the time. On discontinuation of the 4-DOP the nausea and anorexia cleared within 3 days and the patient was able to take the deficient diet for another 30 days. Psychologic reasons seemed to have played a major role in two, for a shift to the thrice-boiled diet alleviated the anorexia in one day. The fourth was an elderly woman who developed anorexia and depression starting on day 20. She did not regain her appetite despite vitamin B_6 supplementation and regular food. These data provide no support for a primary role of pyridoxine in appetite regulation.

DISCUSSION

The wide distribution of vitamin B_6 in natural food products makes induction of vitamin-B_6 deficiency in man by dietetic means a difficult task. To explore the effects of induced vitamin-B_6 deficiency on human neoplastic diseases, other workers depended mainly on the vitamin-B_6 antagonist, 4-DOP, given with a regular or moderately restricted diet. In experimental animal tumors 4-DOP is a relatively ineffective antitumor agent unless it is given with a diet deficient in vitamin B_6. Furthermore, prior to these studies all 4-DOP was synthesized from pyridoxine and, like our drug, probably was contaminated with vitamin B_6. This may have influenced prior results: The feeding of a diet deficient in vitamin B_6 alone was superior to the combination of a diet deficient in vitamin B_6 with supplementation by 4-DOP in causing regression of sarcoma 180 in mice.[11]

In the early part of the present study patients were fed the diet deficient in vitamin B_6 alone. There was definite chemical evidence of vitamin-B_6 depletion but no recognizable effects on patient or tumor. When 4-DOP was added to the deficient diet, toxic side effects consisting of dermatitis and neuritis appeared, but no antitumor effects.

One can conclude that the use of the diet deficient in vitamin B_6, with or without supplementation by 4-DOP, showed no antitumor effect in the spectrum of tumors studied; on the contrary, a possible adverse effect was observed in patients with lymphoproliferative diseases. A rise in peripheral lymphocyte count without change in adenopathy or organomegaly took place in the four patients with lymphoproliferative disease coincidental with or immediately after 4-DOP administration. A similar increase in circulating lymphocytes has been observed in patients with chronic lymphocytic leukemia treated with corticosteroids.[18] The lymphopenic effect of vitamin-B_6 deficiency takes place by a mechanism other than stimulation of the adrenal cortex.[13]

These findings suggest that the treatment might have altered the distribution of lymphocytes between peripheral blood and tissues, despite constancy of lymph node size, or set the disease at a more accelerated phase in some of the patients, possibly by altering immunologic host defenses as observed in Swiss mice with sarcoma 180 during 4-DOP treatment.[10] The rise in circulating lymphocytes in lymphoproliferative disease in man contrasts markedly with the reported lymphopenic effect of vitamin-B_6 deficiency in many animal

species, in particular when the deficiency was induced by 4-DOP.[17] In the present study lymphopenia was demonstrated in patients with non-neoplastic lymphocytes upon the addition of 4-DOP to the deficient diet.

The study confirmed observations made in animal experiments which showed that 4-DOP accentuates some of the manifestations of vitamin-B_6 deficiency. Changes in skin and mucous membrane, neurologic side effects and lymphopenia in patients without lymphoid neoplasms were prominent. Similarly, a significant depression of SGOT and GOT in isolated leukocytes was observed only during periods of 4-DOP administration. There was partial recovery of the enzyme activities upon discontinuation of 4-DOP while continuing on the deficient diet. In a patient with carcinoid syndrome who was included in the study,[4] a drop in platelet serotonin and urinary 5-hydroxyindole acetic acid during 4-DOP administration and partial recovery after the discontinuation of 4-DOP while continuing on the deficient diet was observed.

The changes in hemoglobin percentage, hematocrit and red cell indices and platelet counts were comparable in the patients who received the vitamin-B_6 deficient diet alone and those who received it with 4-DOP. A moderate increase in the reticulocyte counts was observed in 5 of 7 patients during 4-DOP administration, however, in contrast to the reticulocytopenia observed during the period of feeding the deficient diet alone. This observation is similar to that reported by Rosen et al.[17] in which the anemia induced in dogs by feeding a diet deficient in vitamin B_6 was ameliorated upon administration of 4-DOP despite continuous feeding of the diet. No such increase in hemoglobin concentration was observed in our patients but this might be due to the briefer period of 4-DOP treatment.

This finding, together with the increase in leukocyte PLP and urinary pyridoxic acid excretion, prompted a search for possible contamination of the 4-DOP by vitamin B_6. A similar increase in the reticulocyte counts, leukocyte PLP and urinary pyridoxic acid also was observed, however, in some of the patients who received the vitamin B_6-free 4-DOP.

Biochemical evidence of vitamin-B_6 depletion was observed in all patients while fed the deficient diet alone. The addition of 4-DOP to the diet introduced a change in the trend of pyridoxic-acid excretion in the urine and in the PLP levels in leukocytes. Pyridoxic acid usually reappeared in the urine in most of the patients upon institution of 4-DOP treatment. The phenomenon appeared with both batches of 4-DOP. The addition of a known amount of 4-DOP to the urine in vitro did not result in an increase in fluorescence when the procedure for PIC measurement was employed. This does not exclude the possibility that certain products of 4-DOP degradation in vivo might fluoresce in the same range as PIC upon excretion in the urine.

Measurable PLP in the leukocytes often reappeared or increased upon administration of 4-DOP after an initial disappearance or decline while the patients were on the deficient diet alone. The phenomenon was observed with both batches of 4-DOP. This is similar to the increase in liver and leukocyte PLP reported in mice and rats treated with 4-DOP compared to the untreated control.[7, 21] No apparent increase in PLP in liver or muscle was observed in our patients upon receiving 4-DOP. In vitro addition of 4-DOP phosphate to the apotryptophanase enzyme assay did not cause inhibition or enhancement. The findings suggest possible mobilization of PLP from certain depots by the 4-DOP to make it available for the leukocytes and for degradation to PIC. This also could offer a possible explanation for the moderate increase in reticulocyte counts observed during the addition of 4-DOP to the deficient diet.

The findings of fatty infiltration in the liver of three patients while fed the deficient diet was of interest in the light of reported development of fatty infiltraion and eventually cirrhosis in rhesus monkeys fed a diet deficient in vitamin B_6 for a prolonged period of time.[15] The diet fed to the monkeys was well supplemented by lipotropic factors. Our study tends to implicate choline deprivation in the production of these changes.

On the other hand the study implicates the formula diet per se rather than deficiency of vitamin B_6 or choline in the production of the hypocholestremia that the patients developed. Hypo-, hyper- and normocholestremia have been reported by several investigators of vitamin-B_6 deficiency in experiments in animal species.[12] The present data implicate the diet the animals were fed rather than the vitamin-B_6 deficiency per se.

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