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Inhibitory Effect of Pyridoxine Deficiency on Growth of a Transplanted Tumor in Rats.* (31498)

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There have been many attempts to analyze differences in requirements of certain nutritional factors between host and tumor tissue (1,2,3,4). Of special interest have been the studies on vitamins. Major chemotherapeutic advances in the treatment of malignancy have resulted from these studies.

The effect of vitamin B_6 as a constituent that may influence tumor growth has had an interesting but rather enigmatic history. Bischoff *et al* reported the retardation of growth of Sarcoma 180 in mice deficient in pyridoxine; addition of vit. B_6 to the deficient diet caused a significant increase in the growth of the tumor(5). Kline *et al*(6) showed that in rats partially depleted of pyridoxine, the percentage of takes of the Flexner-Jobling carcinoma was lower, number of regressions was higher, and the size of the tumor smaller than in control animals receiving a diet containing pyridoxine and the same number of calories. Similar results were obtained by this group for the Yale adenocarcinoma-1 and a mouse fibrosarcoma. Boutwell *et al*(7) reported that they could lower the incidence of the induction of epithelial tumors in mice only by lowering the concentration of all B vitamins in the diet, and that pyridoxine depletion alone did not affect tumor incidence.

A number of other induced and transplantable tumors have since been observed and evaluated for growth characteristics in hosts made deficient in vit. B_6. In several investigations the pyridoxine antagonist, 4-desoxypyridoxine, was utilized to induce the deficiencies. Results varied according to tumor, host, and experimental procedures(8,9,10,11, 12).

The experiment described here was formulated to demonstrate the relationship of tumor growth to vit. B_6 by quantitating the growth of a transplanted tumor in rats that were made deficient in vit. B_6 by a combination of deficient diet and an antimetabolite regimen.

Materials and methods. Exp. 1. The rats used were Marshall 520 strain obtained from the National Cancer Institute, Bethesda, Md. Eighteen young female animals, ranging in weight from 77 to 122 g, were divided into 3 groups, 2 with 7 animals (deficient group and pair-fed group) and one with 4 animals (*ad libitum* group). The groups of 7 were subdivided into 3 and 4 animals and caged in wire bottom cages so that the collective body weights of the animals in each comparable cage were equal.

The complete diet was as shown in Table I

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TABLE I.

	g/kg	mg/kg	μg/kg
Crude casein (83% protein)	240		
Sucrose	640		
Mazola oil	80		
Salts IV (14)	40		
Vitamins (15)			
Thiamine hydrochloride		4	
Riboflavin		8	
Pyridoxine hydrochloride		4	
Calcium panthothenate		25	
Niacin		40	
Alpha tocopherol		23	
Oleum percomorphum (Mead Johnson)		210	
Menadione		2.1	
B_{12}			300

(13,14). The deficient diet included all ingredients with the exception of vit. B_6.

The deficient group of 3 and 4 animals, respectively, was given the B_6 deficient diet *ad libitum*. After 24 hours the amount of food consumed by the group of animals in each cage was weighed and the same amount of complete diet given to the corresponding rats of the pair-fed group. In addition, to accelerate the deficiency, the deficient animals were given 4-desoxypyridoxine,§ .3 mg/ml (15), in their drinking water *ad libitum*. This was withdrawn at the time of tumor transplant, to avoid any direct toxic effect of the antimetabolite on the tumor. The *ad libitum* group was given a complete diet without restriction. Both pair-fed and *ad libitum* groups were given normal drinking water. All animals were weighed at weekly intervals.

The tumor used was Osteogenic Sarcoma 344, obtained from Dr. Snell, National Cancer Institute(16). This was the 248th transplant generation of this tumor. It grows successfully in 100% of rats after subcutaneous transplant with no regressions; no metastases have been reported.

When it had been determined after a period of about 21 days that a deficiency state had been achieved in the experimental group on the basis of classical changes of acrodynia as described by others(17), 1 mm^3 sections of tumor were cut uniformly by hand and transplanted subcutaneously by trocar into the right inguinal region of the animals. At the same time identical transplants were made into each of the 2 control groups. Ten days later all tumors subsequently measured in this experiment were palpable and by 15 days all were measurable. One animal in each of the 3 groups failed to develop the tumor early enough to include it in the measurements, although all animals eventually did develop the mass. Measurements were taken daily from the 15th to the 22nd day following implantation. The greatest diameter of the mass was measured by placing preformed circular cutouts, of known diameter, over the growth. On the 22nd day after transplant, due to the large size of the tumor in the *ad libitum* group, all animals were sacrificed, the tumors were removed, weighed and examined, and characteristic frozen sections of tumor were taken from each group for microscopic examination with hematoxylin and eosin stain. Every animal was surveyed for metastases, but none were found. Two animals from the deficient group had died in the interim: one on the 17th day and one on the 18th day following transplant. Autopsy revealed an interstitial pneumonia characterized by a mononuclear cell infiltrate.

Exp. 2. This experiment was conducted using the same procedures as described in Exp. 1. Twenty-seven female animals of the Marshall 520 strain were used initially. In this experiment, however, the rats were more mature and of a greater initial weight ranging between 114 g and 164.5 g with the vast majority weighing between 130 and 150 g. With this group it took 24 days before changes of rat acrodynia were seen. Daily measurements of tumor size were taken from the 15th to the 22nd day following transplant. In this experiment, as in the first one, several animals were omitted because tumors failed to appear within the usual time period. Also several animals whose tumors showed retarded growth because of location (*i.e.*, over the sacrum) were omitted.

Results. Exp. 1. Food consumption records revealed that the deficient animals averaged approximately 7 g of food intake per day per animal before the tumor transplant; after transplant daily consumption of

§ Nutritional Biochemicals Corp.

TABLE II. Effect of Pyridoxone Deficiency on Weight of Rats.

	Average weight of animals (g) ± S.D.			
Exp I	Initial	Week 1	Week 2	Week 3
Deficient (7)*	97.2 ± 3.6	95.0 ± 3.1	94.8 ± 2.6	91.0 ± 2.7
Pair-fed (7)	104.6 ± 5.7	105.9 ± 5.3	109.8 ± 5.0	115.7 ± 4.7
Ad libitum (4)	87.6 ± 3.6	92.3 ± 1.4	105.3 ± 2.0	112.6 ± 3.0
Exp II				
Deficient (7)*	139.5 ± 8.7	136.0 ± 7.1	129.0 ± 8.2	121.0 ± 9.9
Pair-fed (6)	142.5 ± 6.8	155.0 ± 11.3	154.0 ± 12.4	166.0 ± 13.9
Ad libitum (7)	139.8 ± 20.5	163.5 ± 34.4	180.9 ± 44.6	195.3 ± 50.2

* Figure in parentheses indicates No. of animals in group.

the deficient animals dropped slightly. The pair-fed group, by contrast, readily consumed its food, often finishing a day's ration within 2 hours.

Table II summarizes the effect of each diet on the growth of the animals prior to implantation of the tumor. While the pair-fed group showed a slight gain in weight and the *ad libitum* group gained moderately (29%), the deficient animals displayed a mild weight loss.

The results presented in Fig. 1 indicate that the tumor in the deficient animals was smaller than that of the pair-fed and *ad libitum* group during the course of the measurements. By termination of the experiment, the neoplasm of the deficient group had not reached the average size that the tumors of the other 2 groups had reached on the first day of measurement 8 days previously. Furthermore, the effects of dietary restriction as seen in the pair-fed animals were not sufficient to account for the retardation of growth. This was substantiated by the fact that the final tumor size of the restricted group was reduced by only 14% over the *ad libitum* controls, while the deficient group showed a reduction of 50% in final tumor size.

Another striking difference may be noted by comparing the final weights of the tumors from each group as shown in Fig. 2. In analyzing these data, however, we must take into account that at autopsy the tumors of the *ad libitum* group were hemorrhagic and necrotic to a marked degree, the neoplasms in the pair-fed group showed moderate necrotic foci, and the deficient group tumors were almost completely free of necrosis. Undoubtedly this necrosis contributed greatly to the final tumor weight of the ad libitum group. However, comparing only the pair-fed with the deficient animals, the average weight of the tumor of the former group is over 3 times greater than that of the deficient group. This comparison of these two groups in which the animals had the same caloric intake, and the diets of which differed by only one constituent (vit. B_6) is revealing.

Comparing the final tumor weight to the final total body weight of the animal (Table III), if we again ignore the normal animals because of the diffuse tumor necrosis, the difference between the neoplasms is noteworthy. The pair-fed tumor accounted for 2.6 times greater percentage of the final body weight than did that of the deficient group.

Histologic sections of tumors from each of the 3 groups revealed no microscopic differences other than those attributable to the increased necrosis. *Exp. 2.* The deficient animals again consumed 7 g of food per day prior to transplant, but following transplant their food consumption increased to 8-9 g per animal per day.

Table II shows weight trends similar to those in Exp. 1. There was a mild weight loss in the deficient animals, a moderate increase in weight in the pair-fed group, and a marked

TABLE III. Effect of Pyridoxine Deficiency on Tumor Weight.

	Tumor weight as % of total body weight ± S.D.	
Diet regimen	Exp I	Exp II
Deficient (4)*	5.0% ± 2.1%	17.3% ± 4.4%
Pair-fed (6)	13.0% ± 1.3%	25.5% ± 3.0%
Ad libitum (3)	25.7% ± 3.6%	21.8% ± 6.4%

* Figure in parentheses indicates No. of animals in group.

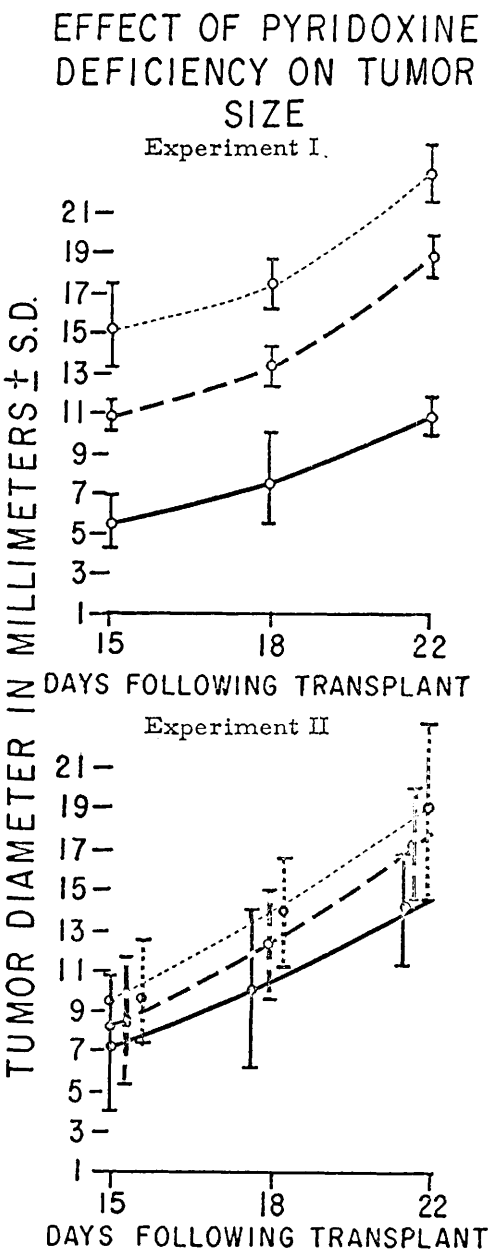

FIG. 1. Average diameter of tumor of deficient animals (lower solid line) as compared to that of pair-fed group (middle broken line) and *ad libitum* group (upper line) over measured period in Exp. I and II.

increase in the *ad libitum* group during the production of the deficiency.

Results presented in Fig. 1 demonstrate that although inhibition of tumor growth occurred it was not as striking as in Exp. 1. Nevertheless, the mean tumor size of the deficient animals was smaller than that of the tumors from the control groups throughout the experiment.

Fig. 2 reveals marked differences in final tumor weight between the deficient animals and the other two groups. Why this difference was not reflected in tumor diameter is not clear. Again the tumors in the deficient animals show far less necrosis than those in the

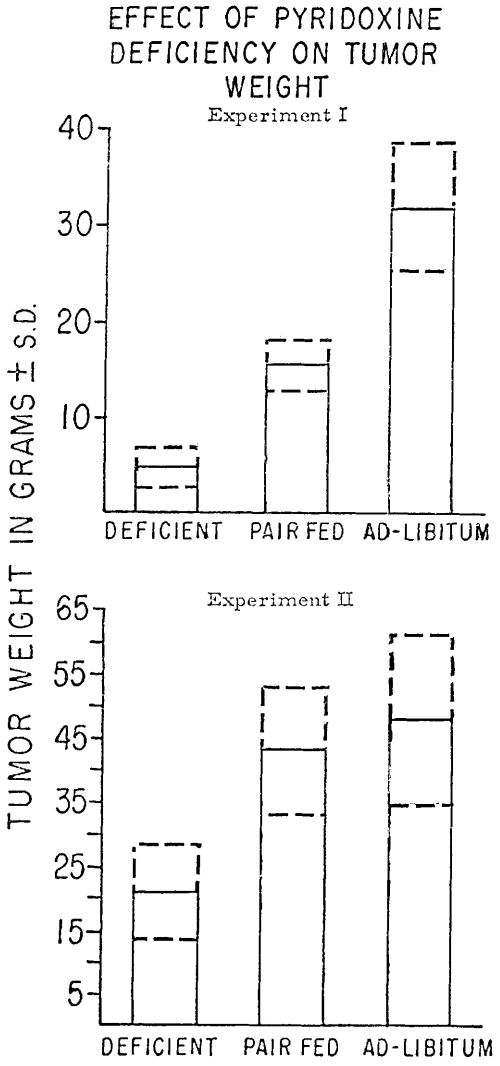

FIG. 2. Comparison of average weights of tumors from the 3 groups. The striking difference in both Exp. I and Exp. II between the deficient group and the other two is readily apparent. However, the difference in tumor necrosis between each group must be considered.

other two groups. Otherwise microscopically no morphological differences in the tumors were seen.

Examination of tumor weight as a percentage of total final body weight (Table III) indicates a difference among the 3 groups not nearly so striking as that in the first experiment. It was surprising to find such a small value for the *ad libitum* group.

Discussion. In recent years much light has been shed on the intricate and vitally important contributions made by pyridoxine in the overall scheme of biochemical processes (18). Its essential role in transamination, plus its integral part in dehydrase, desulfhydrase and decarboxylase reactions show its importance in all aspects of protein metabolism. Vit. B_6 is also known to play a very important role in carbohydrate and fat metabolism(19,20). Much speculation may be made as to the exact role pyridoxine deficiency played in the striking tumor inhibition in Exp. 1. However, subtle factors may play a part. On repeating this protocol in the second experiment with larger, more mature animals, statistically significant effects were not observed though definite trends of tumor inhibition were established (Fig 1). The exact role this difference in maturity plays cannot be definitely stated. Perhaps the older animals had greater tissue storage of vitamin B_6 and were not as depleted as the young rats, or the older animals may not have had as great a need for the available vit. B_6 although tissue storage of the two groups was probably equal. Our criterion for deficiency may have been, therefore, a qualitative phenomenon but not a quantitative one. Mihich and Nichol(10) found that degree of deficiency plays an important role in tumor inhibition. They have shown that inhibition of Sarcoma 180 in mice is related to the length of time the animals were on a B_6 deficient diet prior to transplant of the tumor. Therefore it may be worthwhile to repeat the procedure using quantitative methods to estimate deficiency (*i.e.*, tissue levels of B_6) or semi-quantitative methods (*i.e.*, xanthurenic acid index).

Whether the experimental differences found also signify a possible hormonal relationship is not certain but there is considerable work in the literature regarding the interplay of vit. B_6 and hormones(21). That growth hormone may be an important factor is also speculation. However, Beare and his associates (22) have shown that administration of growth hormone to pyridoxine deficient animals accentuated both qualitative and quantitative manifestations of the deficiencies as measured by skin changes and B_6 content of liver. This latter observation along with the data from the second experiment may also explain the varying results other workers have obtained with vit. B_6 deficiency and other tumor-host systems. Further work is necessary to clarify the precise role that pyridoxine deficiency plays in tumor inhibition, and its possible usefulness as a clinical tool.

Summary. Two experiments were conducted using rats of the Marshall 520 strain depleted in vit. B_6 by the use of a deficient diet and 4-desoxypyridoxine. Osteogenic Sarcoma 344 was transplanted into the deficient animals, a pair-fed group and an *ad libitum* group. Desoxypyridoxine was removed from the diet of the deficient animals on the day of tumor transplant. In Exp. 1 daily measurements of the tumor showed a marked inhibition of the growth of the tumor in the deficient animals as compared to the control groups. The mean weight of the tumor and also the mean weight as a percentage of final mean total body weight were also decreased. In a second experiment larger, more mature animals were used. Tumor inhibition in the deficient animals was again observed, although it was not so striking as in the first experiment. No histological differences in the tumor were noted in either experiment with the exception of increased hemorrhage and necrosis in the neoplasms of the *ad libitum* animals.

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Generalized Aminoaciduria in the Magnesium Deficient Rat.* (31499)

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The experimental induction of magnesium deficiency in the rat has been demonstrated by numerous investigators to cause growth failure, renal damage, hypercalcemia, phosphaturia, urinary nitrogen loss, and kaliuresis (1,2,3). These findings prompted our investigation of urinary amino acid excretion and this report documents the presence of pathologic aminoaciduria and changes in renal sodium-potassium activated Adenosine Tri Phosphatase (Na-K ATPase) in the magnesium deficient rat.‡

Materials and methods. Albino female rats (Holtzman) of initial weight 150-200 g were divided into control and deficient groups. Animals were housed in individual metabolic cages with stool-urine separators in a constant temperature room with 12-hour light cycles. Magnesium deficient diet (General Biochemicals, Chagrin Falls, Ohio), demonstrated to be nutritionally adequate with the exception of magnesium, was homogenized in deionized water and an aliquot of the homogenate representing 7.5 g of the diet was given to each rat twice daily by gavage feeding (15 g diet total daily feeding per rat). With the exception of the addition of 1 mEq magnesium acetate per feeding to control rats, diets were identical for each group. All rats were fed the magnesium-supplemented diet for the initial 6 days to establish baseline values.

Experiment 1: 24 hour urines were collected under toluene, refrigerated, and pooled in 6 day batches for each rat. Stools were collected on a 6 day schedule, weighed, and homogenized in a standard volume of deionized water and refrigerated until analysis. The duration of this experiment was 30 days.

Experiment 2: Control and deficient rats were begun with conditions identical to Exp. 1 except that the magnesium content of the diet of the deficient animals was increased to 0.065 mEq per day to allow a slower onset

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