Acceleration of Growth of Sarcoma 180 with Pyridoxamine and Retardation with Penicillamine.* (28458)

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A study of the vitamin requirement of *Candida albicans* revealed that Vit. B_6 was utilized as a metabolite, shortening the lag phase and reversing the inhibitory action of 5-fluorouracil (5-FU) and 5-fluoro-2'-deoxyuridine (FUDR) noncompetitively, pointing to a new role of this vitamin in nucleic acid metabolism of the organism(1). Growth of the pathogenic yeast was restricted by a number of vitamin antagonists and the inhibition could be reversed by the analogous vitamins for which the organism had requirements(2). To ascertain whether this method of growth regulation and vitamin requirement determination for *C. albicans* could be applied *in vivo*, experiments were begun with Sarcoma 180 in mice using pyridoxamine and one of its little known antagonists, L-penicillamine.

Material and methods. The ascitic form of Sarcoma 180 (S-180) obtained from the Sloan-Kettering Institute for Cancer Research through the courtesy of Dr. K. Sugiura, was maintained in 18 to 22 g, 6-week-old white male CF_1 mice (Carworth, Inc.) by weekly intraperitoneal injections of 1×10^6 ascitic cells/mouse. Solid form S-180 tumors were produced regularly by the single injection of 0.2 ml saline suspension containing 1×10^6 ascitic cells per mouse into the right subcutaneous shoulder region of the animals. Two types of diets were employed, *i.e.*, a complete diet consisting of a standard mouse pellet (Rockland Farms, Inc.) and pyridoxine deficient diet (Nutritional Biochem. Corp.). The mice were housed in stainless steel box-type cages with sawdust bedding and all received diet and water *ad libitum*. 5-FU was obtained from Hoffmann-LaRoche, Inc., pyridoxamine dihydrochloride from Merck, Inc., D-, DL- and L-penicillamine from Nutritional Biochemicals Corp. Mice were weighed every 2 days and at the end of each experiment. The well demarcated solid form sarcomas which developed were excised periodically, weighed and photographed for size.

Results. Accelerating effect of pyridoxamine on ascitic S-180 in mice. Thirty mice previously maintained on a complete diet were divided equally into Groups A, B and C, of which the latter 2 groups received intraperitoneal inoculations of 1×10^6 ascitic S-180 cells/mouse (Fig. 1). Group A remained uninoculated as a nontumor-bearing control. Twenty-four hours later, Group A and tumor-bearing Group C were treated with subcutaneous injections of 15 mg pyridoxamine/mouse daily for 7 days, while Group B remained untreated as the tumor-bearing control. The mice were then rested and observed for 22 days. No mortality was encountered with normal mice which had received 105 mg pyridoxamine over a 7-day treatment period. Administration of pyridoxamine to ascitic S-180-bearing mice (Group C) on the other hand, shortened their life spans (Fig. 1). Life span shortening also occurred in tumor bearing mice given 7.5 and 0.75 mg daily doses of pyridoxamine. This was attributed to the stimulatory effect of pyridoxamine on the ascitic tumor cells.

Reversing effect of pyridoxamine on life prolonging action of 5-FU on ascitic S-180-bearing mice. One hundred and twenty mice maintained previously on a complete diet were divided equally into 4 groups. Group A consisted of a control group of 30 nontumor-bearing normal mice, while the remaining groups were inoculated intraperitoneally with 1×10^6 ascitic cells/mouse (Fig. 2). Forty-eight hours later, Groups A, C and D were treated with 0.5 mg 5-FU intraperitoneally daily for 6 days. Group D received, in addition, daily subcutaneous injections of

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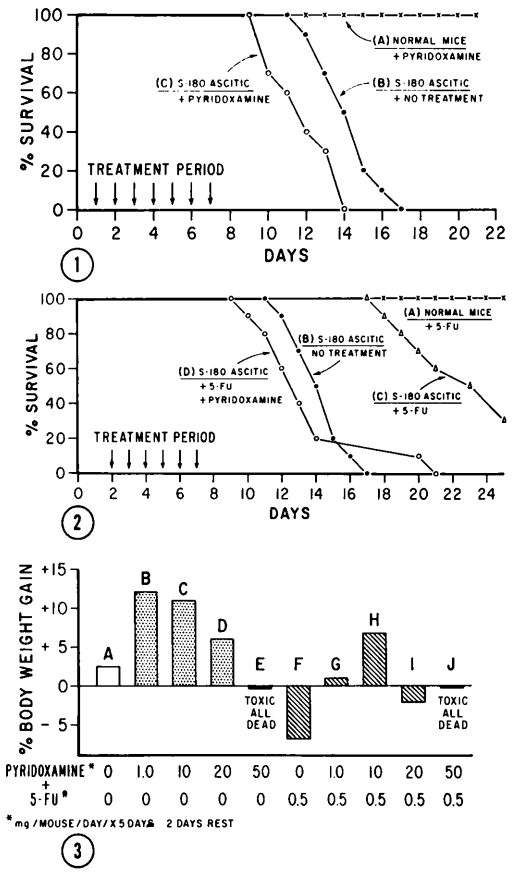


FIG. 1. Accelerating effect of pyridoxamine on ascitic S-180 in mice maintained on complete diet.

FIG. 2. Reversing effect of pyridoxamine on life prolonging action of 5-fluorouracil on ascitic S-180-bearing mice maintained on complete diet.

FIG. 3. Effect of pyridoxamine on weight gain of normal, nontumor bearing mice maintained on complete diet, and reversing effect of pyridoxamine on 5-FU induced weight loss. (5-FU intrap, pyridoxamine subcut, mice weighed on 7th d, 18 mice/group).

0.75 mg pyridoxamine/mouse for 6 days. Group B remained untreated as a tumor-bearing control. Following treatment, all mice were rested from the 8th to 25th day and fed *ad libitum*. Nontumor-bearing normal mice (Group A), tolerated treatment with six 0.5 mg doses of 5-FU without mortality (Fig. 2). Also, intraperitoneal administration of 5-FU to ascitic S-180-bearing mice (Group C) prolonged their life span in comparison with the untreated tumor-bearing Group B. However, the concomitant subcutaneous injections of pyridoxamine with 5-FU to tumor-bearing Group D reversed this life prolonging action (Fig. 2). This also occurred with 1.0, 7.5 and 15 mg daily doses of pyridoxamine administered concomitantly with 5-FU.

Effect of pyridoxamine on weight gain of normal nontumor-bearing mice maintained on complete diet and reversing effect of pyridoxamine on 5-FU-induced weight loss. Untreated normal mice maintained on a complete diet gained 2.5% in body weight at the end of 7 days (Group A) (Fig. 3). Subcutaneous administration of pyridoxamine to to Groups B, C and D caused significant increase in body weight over that of the control group. 5-FU administration alone caused 7% body weight loss in Group F, while increasing doses of pyridoxamine nullified this effect in 5-FU-treated Groups G, H, and I. Five successive daily doses of 50 mg pyridoxamine were toxic to both untreated and 5-FU-treated mice. Since all mice, including those treated with 5-FU had been maintained on a complete diet which contains some Vit. B_6, and this would exert some reversing action on 5-FU, pyridoxine deficient diet was next introduced to eliminate this factor.

Accelerating effect of pyridoxamine on growth of solid form S-180 in mice maintained on pyridoxine deficient diet. Subcutaneous administration of 10 mg pyridoxamine daily for 11 days to mice on pyridoxine deficient diet increased their average body weight 35%, while it more than doubled the weight of the subcutaneous solid form sarcomas (Table I). This is more increase in tumor weight than can be attributed solely

TABLE I. Accelerating Effect of Pyridoxamine on Growth of Solid Form Sarcoma 180 in CF_1 Mice Maintained on Pyridoxine Deficient Diet.*

Pyridoxamine daily dose (i.p.) mg	No. of mice	Avg change in body wt† g	Avg change in body wt† %	Avg tumor wt ± S.D. (mg)
0	10	+ .7	+ 3.5	700 ± 26
.1	10	+2.8	+14.0	1400 ± 37
1.0	10	+5.5	+27.5	1900 ± 43
10.0	10	+7.0	+35.0	1900 ± 43

* Maintained on diet for 16 days, followed by subcutaneous implantation of 1×10^6 ascitic cells/mouse and continuation of diet for 12 more days. Pyridoxamine therapy 24 hr after tumor implantation and daily for 11 days. Average initial wt 20 g, after 16 days of diet, 19 g.

† Beginning from day of tumor implantation.

TABLE II. Comparison of Normal Diet, Pyridoxine Deficient Diet, and Deficient Diet Enriched with Pyridoxamine, on Growth of Solid Form Sarcoma 180.*

Diet	Days on diet after tumor implant	No. of mice	Avg change in body wt† g	Avg change in body wt† %	Avg tumor wt ± S.D. (mg)	Tumor inhibition (%)
Complete‡	8	10	+2.4	+10.8	800 ± 28	
"	12	10	+3.6	+15.6	1400 ± 37	
Pyridoxine deficient§	8	10	+1.4	+ 7.1	300 ± 17	63
" "	12	10	+ .7	+ 3.5	700 ± 26	50
Pyridoxine deficient + pyridoxamine‖	8	10	+2.0	+ 8.2	1100 ± 33	0
Idem	12	10	+3.6	+15.0	2200 ± 47	0

* All mice maintained on respective diets for 16 days, followed by subcut implantation of 1×10^6 ascitic cells/mouse, and continuation of diet as above.
† Beginning from day of tumor implantation.
‡ Standard pellet, Rockland Farms, Inc.
§ Nutritional Biochem. Corp.
‖ 1 mg pyridoxamine/100 g pyridoxine deficient diet.

to the weight gain of the host, and was taken to indicate the heightened avidity for pyridoxamine of solid S-180 tissue over that of normal tissue.

Comparison of complete diet, pyridoxine deficient diet and pyridoxine deficient diet enriched with pyridoxamine on growth of solid form S-180. Three groups of 20 mice each, maintained on 3 different diets for 16 days, all received subcutaneous implantations of 1×10^6 ascitic S-180 cells/mouse, and were continued on their respective diets. At 8 days after tumor implantation, solid sarcomas were one-half the weight in mice on pyridoxine deficient diet as in those on the complete diet (Table II). By the 12th day, sarcomas in mice on the deficient diet were again ½ the weight as on the complete diet. The tumor retarding effect was attributed to B_6 lack since admixture of pyridoxamine to pyridoxine deficient diet not only completely nullified the tumor retarding effect of the deficient diet, but produced tumors actually larger and heavier in weight than in mice on complete diet (Table II). The data indicate that pyridoxamine exerts a pronounced growth stimulatory effect on both ascitic and solid form S-180 in CF_1 mice, that growth of sarcoma is retarded by pyridoxine deficient diet, and that pyridoxamine added to deficient diet nullifies this effect. If it were now possible to reproduce chemotherapeutically the regressions induced by pyridoxine deficient diet or to reinforce this effect with a B_6 antagonist, it would help confirm the requirement of S-180 for Vit. B_6 and contribute to the control of this tumor system. A compound selected for this possible action was L-penicillamine since it had been observed that addition of the compound to the diet of albino rats increased the animal's requirement for Vit. B_6 and produced the appearance of pyridoxine deficiency symptoms (3). *Retarding effect of L-penicillamine on growth of solid form S-180 in mice maintained on a complete diet.* Mice bearing solid form S-180 gained 24.5% in body weight when maintained on complete diet for 3 weeks, while the implanted sarcomas increased 7-fold in weight (Table III). L-penicillamine-treated mice, on the other hand, showed sarcoma weight increase of only 3-fold, or 60 to 72% inhibition over that of the untreated mice. Average body weight gain in the L-penicillamine-treated group was minimal (4.5%) and at the end of 2 weeks of therapy many mice exhibited roughened coats and other signs of pyridoxine deficiency (Fig. 4, 5). *Retarding effect of L-penicillamine on growth of solid form S-180 in mice maintained on pyridoxine deficient diet and the reversing effect of pyridoxamine.* L-penicillamine treatment caused pronounced retarding effect on the growth of solid form S-180 in both pyridoxine deficient diet as well as the same diet enriched with pyridoxamine (Table IV). The average weight of 7-day-old solid sarcomas in mice on deficient diet and treated with L-penicillamine was one-fifth that in mice on deficient diet alone. Enrichment of

TABLE III. Retarding Effect of L-Penicillamine on Growth of Solid Form Sarcoma 180 in CF_1 Mice Maintained on Complete Diet.*

Treatment	Weeks of therapy	Avg change in body wt† g	Avg change in body wt† %	Avg tumor wt ± S.D. (mg)	Mortality
None (control)	1	+1.7	+ 8.5	500 ± 22	0/10
	2	+3.5	+17.5	1900 ± 43	0/10
	3	+4.9	+24.5	3450 ± 58	0/10
L-penicillamine 1 mg/mouse/day/ × 14 days, subcut	1	+1.2	+ 6.0	300 ± 17	0/10
	2	+1.9	+ 8.5	760 ± 27	0/10
	3‡	+ .9	+ 4.5	970 ± 31	0/10

* All mice received subcut implantation 1 × 10^6 ascitic cells/mouse at start.
† Beginning from day of tumor implantation.
‡ 7 day rest, no L-penicillamine.

TABLE IV. Retarding Effect of L-Penicillamine on Growth of Solid Form Sarcoma 180 in CF_1 Mice Maintained on Pyridoxine Deficient Diet and Nullifying Effect of Pyridoxamine on Retardation.*

Diet	Group	L-penicillamine mg/mouse/day × 7 days, s.c.	Avg change in body wt† g	Avg change in body wt† %	Avg tumor wt ± S.D. (mg)	Tumor inhib (%)	Mortality
Pyridoxine deficient	A	0	+1.4	+ 7.4	300 ± 17		0/10
	B	.5	−1.2	− 6.3	180 ± 40	40	7/10
	C	1.0	−1.5	− 7.9	60 ± 8	80	8/10
Pyridoxine deficient + pyridoxamine‡	D	0	+2.0	+ 8.5	1100 ± 33		0/10
	E	.5	+2.5	+11.0	1050 ± 32	6	0/10
	F	1.0	+1.2	+ 5.5	750 ± 27	33	3/10

* All mice maintained on their diets for 16 days, followed by subcut implantation of 1 × 10^6 ascitic cells/mouse.
† Beginning from day of tumor implantation.
‡ 1 mg pyridoxamine/100 g pyridoxine deficient diet.

the deficient diet with pyridoxamine completely nullified the tumor-retarding effect of the deficient diet but only partly nullified the tumor-retarding effect of L-penicillamine. This would imply that there is a second mechanism, other than B_6 antagonism, responsible for L-penicillamine tumor retarding action. Judging from the nature of L-penicillamine, it is probably chelation. The data clearly indicate (1) pyridoxine deficient diet, although toxic to a degree, exerts a significant retarding effect on the growth of solid form S-180(4,5,6), (2) L-penicillamine adds to the tumor retarding effect of this diet, (3) pyridoxamine added to pyridoxine deficient diet nullifies the tumor retarding effect of the diet, as well as some of the tumor retarding effect of L-penicillamine, and (4) pyridoxamine and L-penicillamine act *in vivo* as a vitamin-vitamin antagonist pair. The implications of this type of antagonism and the possible use of other vitamin deficient diets and vitamin antagonists for control of tumor growth other than, and including S-180, makes further investigation important. *Comparative growth retarding effect of 5-FU and L-penicillamine on solid form S-180 in mice maintained on pyridoxine deficient diet.* Pyridoxine deficient diet alone exerted significant tumor retarding effect when compared with complete diet (Table V). Reinforcement of deficient diet with 5-FU caused 51% additional tumor inhibition, while reinforcement with L-penicillamine caused 59% tumor inhibition. Concomitant administration of both compounds did not produce additive effects. L-penicillamine was as effective as 5-FU in retarding the growth of solid form S-180 in mice on pyridoxine deficient diet and was less toxic. *Growth retarding effect of L-penicillamine on solid form S-180 in mice despite delayed therapy.* Treat-

ment of mice carrying 4-day-old matured solid form S-180 subcutaneous implants, with 4 successive intraperitoneal doses of L-penicillamine had no inhibitory effect on sarcoma development by the 8th day in mice maintained on complete diet (Table VI). However, with pyridoxine deficient diet reinforced with L-penicillamine there was 68% tumor inhibition over the control, and 31% with pyridoxine deficient diet alone, despite the 96-hour delay in therapy. Doses of L-penicillamine were higher than tolerated in this experiment, since there was 14% loss in body weight and 33% mortality at the end of the experiment. *Growth retarding effect of L-, D-, and DL-penicillamine on solid form S-180 in mice maintained on complete diet.* Forty mice maintained on a complete diet received subcutaneous implantations of 1×10^6 ascites S-180 cells/mouse and were divided equally into 4 groups. The following day, intraperitoneal injections of 1 mg each of L-, D- and DL-penicillamine per mouse were administered separately to each animal group daily for 14 days. The mice were sacrificed on the 15th day and solid sarcomas excised and weighed. L-penicillamine produced 55% tumor inhibition over that of the untreated animals, while D- and DL- forms were less active (Table VII). Mice treated with L-penicillamine for 14 days showed some signs of Vit. B_6 deficiency and there was no mortality.

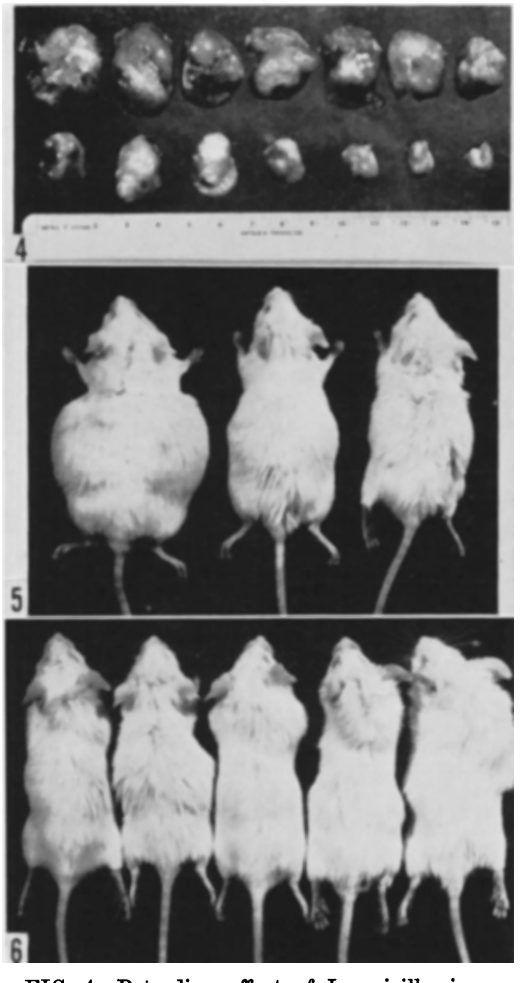


FIG. 4. Retarding effect of L-penicillamine on growth of solid form S-180 in mice maintained on complete diet. Sarcomas excised 3 weeks after subcut implantation of 1×10^6 ascitic cells/mouse. TOP ROW: No treatment BOTTOM ROW: 1 mg L-penicillamine/mouse/day/ $\times$ 14 days, plus 7 days without treatment.

FIG. 5. Retarding effect of pyridoxine deficient diet and L-penicillamine on development of ascitic S-180 in mice, 9 days after intrap inoc 1×10^6 ascitic cells/mouse. LEFT: *complete diet* MIDDLE: *pyridoxine deficient diet* RIGHT: *pyridoxine deficient diet + L-penicillamine* (1 mg/mouse/day/ $\times$ 9 days).

FIG. 6. Effect on development of solid form S-180 of L-penicillamine and pyridoxamine in mice maintained on pyridoxine deficient diet. All mice implanted subcut in rt shoulder region 9 days previously with ascitic tumor cells. FROM LEFT TO RIGHT: 1st: deficient diet alone, 2nd: + L-penicillamine, 3rd to 5th: increasing doses of pyridoxamine (0.1, 1.0 and 10 mg/mouse/day/ $\times$ 9 d).

Discussion. Eighteen normal and malignant human cell lines cultured in semi-synthetic substrates showed an essential requirement for 8 vitamins, including pyridoxine(7). A similar requirement for pyridoxine was indicated by the retardation of growth of induced and transplantable tumors in animals deprived of this vitamin(4-6). The presence of pyridoxal and pyridoxal phosphate in the vicinity of isolated Ehrlich ascites tumor cells intensified their uptake of amino acids both under aerobic(8) and anaerobic conditions (9). These tumor cells quickly took up pyridoxal calculated to be 1.3 to 1.4 times that of the extracellular level of the vitamin(10). Coincidentally, Ehrlich ascites tumor cells appear to have a greater capacity to take up amino acids from the blood and concentrate them than do normal cells(11). These findings are all compatible with the known primary functions of Vit. B_6 as a coenzyme in amino acid decarboxylation, transamination,

TABLE V. Comparative Growth Retarding Effect of 5-Fluorouracil and L-Penicillamine on Solid Form Sarcoma 180 in CF_1 Mice Maintained on Pyridoxine Deficient Diet.*

Diet	5-FU‡ i.p. (mg)	L-Pen‡ i.p. (mg)	Avg change in body wt† g	Avg change in body wt† %	Avg tumor wt ± S.D. (mg)	Tumor inhib (%)	Mortality
Pyridoxine deficient	0	0	+3.5	+12.9	850 ± 29		0/7
Idem	.5	0	−2.3	− 9.5	420 ± 21	51	2/6
"	0	1.0	−2.3	− 9.3	350 ± 18	59	2/6
"	.5	1.0	−3.2	−13.3	360 ± 18	58	2/6
Complete diet (control)	0	0	+4.1	+15.2	1250 ± 35	0	0/10

* All mice maintained on their diets for 13 days, followed by subcut implantation of 1×10^6 ascitic cells/mouse. Drug therapy commenced 24 hr later and daily for 8 days, sarcomas excised 9th day.
† Beginning from day of tumor implantation.
‡ Per mouse/day/ × 8 days.

TABLE VI. Retarding Effect of L-Penicillamine on Growth of Solid Form Sarcoma 180 in CF_1 Mice Despite Delay in Therapy* of 96 Hours.

Diet	Group	L-Penicill i.p. daily dose (mg)	Avg change in body wt† g	Avg change in body wt† %	Avg tumor wt ± S.D. (mg)	Tumor inhib (%)	Mortality
Complete	A	0	+5.5	+26.1	1640 ± 40		0/12
"	B	5,2,2,2	+3.0	+14.6	1630 ± 40	0	2/12
Pyridoxine deficient	C	0	+ .5	+ 2.3	1130 ± 33	31	0/12
" "	D	5,2,2,2	−3.0	−14.5	530 ± 33	68	4/12

* All mice maintained on complete diet, followed by subcut implantation 1×10^6 ascitic cells/mouse, then divided into treatment groups. L-penicillamine on 4th, 5th, 7th and 8th days, sarcomas excised on 10th day.
† Beginning from day of tumor implantation.

TABLE VII. Retarding Effect of Penicillamine Isomers on Growth of Solid Form Sarcoma 180 in CF_1 Mice Maintained on Complete Diet.*

Penicillamine isomer 1 mg/ mouse/day/14 days (i.p.)	Group	Avg change in body wt† g	Avg change in body wt† %	Avg tumor wt ± S.D. (mg)	Tumor inhibition (%)	Mortality
No therapy	A	+3.1	+13.0	4900 ± 70	0	0/10
L-form	B	+6.2	+27.0	2200 ± 47	55	0/10
D-form	C	+8.9	+38.8	2900 ± 54	41	0/10
DL-form	D	+6.6	+28.7	3800 ± 62	22	0/10

* Subcut tumor implantation of 1×10^6 ascitic cells/mouse 24 hr before therapy with penicillamine. Sarcomas excised on 15th day.
† Beginning from day of tumor implantation.

serine and threonine dehydrase and cysteine desulfhydrase reactions and in the catalytic breakdown of tryptophan by tryptophanase. It was not surprising therefore, to encounter the stimulatory action of pyridoxamine on the *in vivo* development of ascitic and solid form S-180 following injection of the vitamin or addition to the diet.

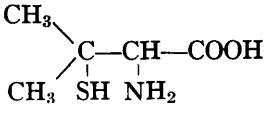
In treating S-180-bearing mice with pyridoxine deficient diet and reinforcing it with L-penicillamine, a B_6 antagonist of low toxicity, significant retardation of sarcoma growth occurred. The tumor retarding effect of pyridoxine deficient diet is undoubtedly due to impairment of those cell functions dependent upon Vit. B_6, since addition of pyridoxamine to the diet as well as parenteral administration of the vitamin, nullified this effect. Subcutaneous administration of pyridoxamine to S-180-bearing mice on deficient diet increased average body weight 35%, while it almost tripled the weight of the sar-

comas. The heightened avidity of S-180 tumor tissue for pyridoxamine thus provides an exploitable biochemical difference between it and normal tissue (Fig. 6).

Several investigators have demonstrated activity of pyridoxine antagonists such as 4-deoxypyridoxine and isonicotinic acid hydrazide against transplantable tumors grown in pyridoxine deficient animals(12-16). However, these antagonists were ineffective in animals maintained on a complete diet(16), indicating that their action was easily nullified by the corresponding vitamin in the diet. In the studies herein reported, L-penicillamine exerted a definite tumor retarding effect in mice despite their maintenance on a complete diet. Retardation of S-180 and 3 other transplantable tumors in pyridoxine deficient animals was first reported in 1943(17). In animals fed pyridoxine deficient diet, there was inhibition to a varying degree of the growth of Adenocarcinoma 755, Ehrlich carcinoma (solid), Murphy-Sturm lymphosarcoma, Leukemias 1210 and L4946, Ehrlich carcinoma clone 2 (ascites) and Plasma-cell tumor 70429(18). Since this infers that these tumor types require Vit. B_6, in view of our results, we would surmise that L-penicillamine would be similarly effective against these tumor types. It remains to be seen whether L-penicillamine, with or without pyridoxine deficient diet fed at sub-toxic levels, will have tumor retarding potential in B_6-responsive tumors in humans. The requirement of malignant human cell lines for pyridoxine, the growth retarding effect of pyridoxine deficient diet on many transplantable tumors, and our demonstration of the pronounced stimulatory effect of pyridoxamine on the *in vivo* development of S-180, suggest that Vit. B_6 and its congeners be withheld from parenteral or oral administration to tumor patients unless their neoplasms are known not to be B_6-responsive.

The solid form S-180 tumor, one of the oldest of transplantable tumors in existence and originally a mammary carcinoma, is not very responsive to chemotherapeutic agents. Only 17 of 122 compounds, including 5-FU but not penicillin, were found to exert inhibitory effects(19). 5-FU therapy reinforced the tumor retarding effect of pyridoxine deficient diet on the growth of solid form S-180 and caused a life prolonging action on ascitic S-180-bearing mice. Pyridoxamine reversed this effect and also reversed 5-FU-induced weight loss in normal mice. The *in vivo* antagonism of pyridoxamine with 5-FU reinforces the possibility that B_6 is involved in nucleic acid metabolism.

L-penicillamine (β-mercaptovaline,β,β-dimethyl-cysteine) was chosen for chemotherapeutic trial because of its antagonist activity to Vit. B_6 and the sensitivity of S-180 to pyridoxine dietary deficiency. The compound has not been used as a tumor retarding agent before, to our knowledge. DL-penicillamine and other penicillin derivatives were reported as inactive against S-180 in the 1953 Negative Data from Experimental Cancer Chemotherapy Studies (Cancer Research Supplement No. 1). It appears as a urinary excretory product following parenteral administration of penicillin(20). It is a nontoxic, water soluble and acid-stable amino acid that is active orally in daily doses of 900 mg for treatment of hepatolenticular degeneration (Wilson's disease), lead poisoning and hemosiderosis because of its pronounced chelating action(20,21). Penicillamine is similar to cysteine in many respects but differs in being more water soluble and in not being oxidized by enzymes occurring in animal tissues. Natural penicillamine



belongs to the D-series. The antagonistic action of L-penicillamine against B_6 *in vivo* has been attributed to its action in increasing excretion of Vit. B_6 and to its chemical interaction with pyridoxal or pyridoxal phosphate (22). The antagonism may well be due to other mechanisms involving B_6 since pyridoxine deficiency in rats disturbs sodium and potassium tissue level(23), decreases I^{131} uptake in the thyroid gland(24) and decreases urinary excretion of manganese(25). Desoxypyridoxine increases serum magnesium in rabbits(26). The failure of pyridoxamine

completely to nullify the tumor retarding effect of L-penicillamine on solid S-180 in mice on pyridoxine deficient diet suggests that the tumor retarding action of L-penicillamine is due to some other factor besides its antagonism to Vit. B_6, probably chelation.

Summary. Pyridoxamine dihydrochloride accelerated the growth of ascitic Sarcoma 180 in CF_1 mice and shortened the life span of these tumor bearing animals. It also accelerated the growth of the solid form of the tumor, indicating the strong B_6-avidity of this tumor system. Pyridoxine dietary deficiency alone retarded the growth of the solid form of the tumor, while pyridoxamine nullified this effect. A relatively nontoxic B_6 antagonist, L-penicillamine (β-mercaptovaline), used clinically as a chelating agent, was found to retard the growth of solid form S-180 in mice when they were maintained on a complete diet, and especially so on pyridoxine deficient diet. The L-, D- and DL-forms of penicillamine were all active as sarcoma retarding agents, the L-form being the most active. Growth retardation of solid form S-180 was produced by L-penicillamine whether therapy was started 24 hours after tumor implantation or was delayed for 96 hours. L-penicillamine was equally as active as 5-fluorouracil in retarding the growth of solid form S-180 in mice maintained on pyridoxine deficient diet and was less toxic. The tumor retarding effect of L-penicillamine was partly nullified by pyridoxamine, the two compounds acting *in vivo* as a vitamin and antivitamin pair. Pyridoxamine reversed the life prolonging action of 5-fluorouracil on ascitic S-180-bearing mice and reversed 5-fluorouracil induced weight loss in normal mice. Therapy of a B_6-responsive transplantable tumor in mice such as Sarcoma 180, with the use of pyridoxine deficient diet plus administration of L-penicillamine was successful in significantly retarding the *in vivo* development of the tumor.

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