The Effect of Pyridoxine Deficiency on a Spectrum of Mouse and Rat Tumors*

ENRICO MIHICH, FRED ROSEN, AND CHARLES A. NICHOL

(Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo 3, N.Y.)

In the initial studies on the effect of pyridoxine deficiency upon tumor growth, deficient diets were fed to rats and mice to impair the functions dependent upon the vitamin; inhibition of tumor growth was observed with Sarcoma 180 (S-180) (1), Flexner-Jobling carcinoma, Yale adenocarcinoma-1 and a mouse fibrosarcoma (7). In nearly all subsequent investigations dietary depletion was combined with the administration of an antimetabolite, usually 4-deoxypyridoxine (4-DOP), and in many instances reduction in the growth of tumors was observed (2, 8, 12-14). The effects produced by pyridoxine deficiency are not, however, necessarily the same as those resulting from treatment with antagonists of this vitamin, since qualitative differences in the effects on certain enzymes have been reported (6).

Recent studies in this laboratory demonstrated that the growth of S-180 was impaired in mice fed a pyridoxine-deficient diet for 2 weeks or longer prior to implantation; furthermore, a considerable number of tumors regressed completely under these conditions (9). Complete regression of established S-180 acquired particular significance, since this tumor, although sensitive to a number of agents, had been found to regress completely only following treatment with 6-mercaptopurine (4).

The study of the effects of pyridoxine deficiency alone on a limited spectrum of tumors was therefore considered desirable. Growth of Adenocarcinoma 755, Ehrlich carcinoma (solid), Murphy-Sturm lymphosarcoma (MSL), Walker carcinosarcoma 256 (W-256), Ehrlich carcinoma solid (E-ca), and Ridgway osteogenic sarcoma (ROS) from Dr. K. Sugiura, Sloan-Kettering Institute; Adenocarcinoma 755 (Ad-755) from Dr. H. E. Skipper, Southern Research Institute; Leukemias L1210 and L4946 from Dr. L. W. Law, National Cancer Institute; Plasma-cell tumor 70429 (P70429) from Dr. M. Potter, National Cancer Institute; and Ehrlich ascitic carcinoma clone 2 (E-2) from Dr. J. F. Holland of this Institute. Sarcoma 180 (S-180), E-ca and E-2 were grown in Ha ICR Swiss mice; L1210 in DBA/2 mice; ROS and L4946 in AKR mice; Ad-755 in C37BL/6 mice, and P70429 in C3H mice. W-256 and MSL were grown in Sprague-Dawley rats obtained from the Holtzman Company. Female rodents were used in all cases.

The transplantation technics and procedures for the evaluation of tumor growth were as previously described for solid and ascitic Sarcoma 180 (9). The mice were kept in shoe box-type cages with sawdust bedding and the rats in suspended wire-bottom cages. The purified sucrose-casein diets (complete and pyridoxine deficient), described previously (9), were fed ad libitum.

RESULTS

The growth of five ascitic tumors was impaired in mice fed the deficient diet as indicated by cell counts (Table 1). The results obtained previously with Sarcoma 180 ascites (9) are reported for comparative purposes. Although some tumors were inhibited by as much as 60 per cent, in no instance was an increase of survival of the deficient animals observed.

The growth of Sarcoma 180 was impaired least among these ascitic tumors; this was unexpected in view of the striking inhibition of solid Sarcoma 180 under similar conditions (9). Subsequently, the growth of solid and ascitic S-180, both inoculated...
subcutaneously, was compared in pyridoxine-deficient mice (Table 2). The impairment of growth and the regressions of solid S-180 in this nutritional condition were confirmed; the "ascitic" S-180, however, was not inhibited initially. Six weeks after implantation, many of the mice bearing the "ascitic" tumor were still alive; at this point the tumors in the deficient animals were 70 per cent smaller (av. diam., 9 mm.) than those in the mice fed the complete diet (av. diam., 32 mm.). The inhibited "ascitic" tumors, however, did not regress.

Others have reported that the growth of Ad-755 was inhibited by DOP in mice fed a pyridoxine-deficient diet from the day of implantation (12). More recently, the same tumor was found to regress in animals treated with a combination of agents, one of which was DOP (11). Experiments were designed, therefore, to ascertain whether this tumor would regress in mice fed the pyridoxine-deficient diet alone. The growth of the tumor was significantly inhibited in mice fed the deficient diet starting 2-7 weeks prior to implantation; this effect could not be attributed to any significant loss of body weight (Table 3). Four weeks after implantation, inhibition of tumor growth ranged from 65 to 100 per cent but was then accompanied by severe toxicity and a high incidence of mor-

### Table 1

**Effect of Pyridoxine Deficiency on Five Mouse Ascitic Tumors**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Period of Deficiency Prior to Inoculation (weeks)</th>
<th>Leukemias</th>
<th>Ehrlich Ascitic Carcinoma Clone 2</th>
<th>Plasma-cell Tumor 7049</th>
<th>Sarcoma 180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L1210</td>
<td>L4946</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibition (per cent)</td>
<td>Av. survival (days)</td>
<td>Inhibition (per cent)</td>
<td>Av. survival (days)</td>
</tr>
<tr>
<td>Complete</td>
<td></td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Pyridoxine-deficient</td>
<td></td>
<td>1</td>
<td>8</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td></td>
<td>2</td>
<td>8</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td></td>
<td>4</td>
<td>9</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td></td>
<td>7</td>
<td>9</td>
<td>50</td>
<td>13</td>
</tr>
</tbody>
</table>

* Average values are presented for two to three experiments in each of which 25 mice per group were kept for evaluation of survival time, and five mice per group were used for cell counts.
† The per cent inhibition is based on cell counts performed on the 5th day (L1210 and L4946), on the 6th day (Ehrlich clone 2), on the 10th day (S-180) and on the 14th day (plasma-cell tumor) after inoculation.
‡ The deficient diet was given starting on the day of implantation.

### Table 2

**Comparative Effects of Pyridoxine Deficiency Against Ascitic and Solid Sarcoma 180**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Origin of the Tumor</th>
<th>No. Mice</th>
<th>8th Day after Implantation</th>
<th>15th Day after Implantation</th>
<th>6th Week after Implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Av. Δ† weight (gm.)</td>
<td>Mortality (no.)</td>
<td>Av. tumor diam. ± S.D. (mm.)</td>
</tr>
<tr>
<td>Complete Deficient‡</td>
<td>Solid S-180</td>
<td>10</td>
<td>-1.0</td>
<td>0</td>
<td>11.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>-0.6</td>
<td>0</td>
<td>4.3 ± 1.1</td>
</tr>
<tr>
<td>Complete Deficient‡</td>
<td>Ascitic S-180</td>
<td>10</td>
<td>+2.0</td>
<td>0</td>
<td>7.4 ± 1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>-0.8</td>
<td>0</td>
<td>6.5 ± 2.9</td>
</tr>
</tbody>
</table>

* Both tumors were implanted subcutaneously. The inoculum for the ascitic tumor was 1 × 10⁶ cells per mouse.
† Average change in body weight from that on day of implantation.
‡ The deficient diet was fed for 3 weeks prior to implantation of tumors.
tality (32 to 89 per cent). A certain number of tumors regressed completely 3–7 weeks after implantation. Most of the regressions occurred with loss of weight of less than 10 per cent of the initial weight. Severe loss of body weight was seen, however, at the time of death. Very few of the tumor-free deficient mice survived longer than the tumor-bearing controls (Chart 1).

Growth of Ehrlich carcinoma (solid) was only slightly impaired in the deficient mice (two experiments); no significant tumor inhibition was seen at the end of the second week of growth. On the 28th day after implantation, however, 35–52 per cent inhibition was observed. Sixteen per cent of the tumors grown in the deficient animals were extruded by ulceration, whereas all the completely fed mice died bearing their tumor. No significant impairment of the growth of Ridgway osteogenic sarcoma was found in pyridoxine-deficient AKR mice (two experiments).

**TABLE 3**

**Effect of Pyridoxine Deficiency on the Growth of Adenocarcinoma 755**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Period of deficiency prior to implantation (weeks)</th>
<th>No. mice</th>
<th>15th Day after implantation</th>
<th>Av. Δ weight* (gm.)</th>
<th>Mortality (per cent)</th>
<th>Av. Tumor diam. ± S.D. (mm.)</th>
<th>Inhibition (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>-</td>
<td>69</td>
<td></td>
<td>-0.9</td>
<td>7</td>
<td>7.7 ± 3.5</td>
<td>-</td>
</tr>
<tr>
<td>Pyridoxine-deficient</td>
<td></td>
<td></td>
<td></td>
<td>0.0</td>
<td>2</td>
<td>7.8 ± 3.3</td>
<td>6</td>
</tr>
<tr>
<td>&quot;</td>
<td>2</td>
<td>56</td>
<td></td>
<td>-0.1</td>
<td>0</td>
<td>4.5 ± 3.4</td>
<td>42</td>
</tr>
<tr>
<td>&quot;</td>
<td>4</td>
<td>50</td>
<td></td>
<td>-1.5</td>
<td>8</td>
<td>5.6 ± 3.5</td>
<td>28</td>
</tr>
<tr>
<td>&quot;</td>
<td>7</td>
<td>34</td>
<td></td>
<td>+0.6</td>
<td>24</td>
<td>2.5 ± 2.9</td>
<td>68</td>
</tr>
</tbody>
</table>

* Average change in body weight from that on day of implantation (initial weight, 18–22 gm.).
† The deficient diet was given starting on the day of implantation.

The growth of Walker carinosarcoma 256 was not significantly affected in the deficient animals. Murphy-Sturm lymphosarcoma was not significantly inhibited (evaluation made on the 15th day after implantation) when grown in rats fed the deficient diet starting 2 weeks prior to, or on the day of implantation; 46–65 per cent inhibition, respectively, was seen in rats fed the deficient diet starting 4 and 7 weeks prior to implantation. In some of the deficient rats this tumor regressed, but this effect could not be attributed to the deficiency of the animals, since approximately the same percentage of regressions occurred in the control rats.

The antitumor effects of pyridoxine deficiency and the toxicity of this diet in the different strains of mice used are summarized in Table 4. The data indicate that Swiss, DBA/2, and C3H mice are less sensitive to the toxic effects of pyridoxine deficiency than C57BL/6 and AKR mice in the nutritional condition which caused the maximum tumor inhibition. It should be mentioned that C3H mice are as sensitive as the AKR mice to the deficient diet fed starting 7 weeks prior to implantation. In this condition, however, the inhibition of growth of the P7049 tumor is not greater than that seen in mice depleted for 2 weeks (see also Table 1). All the tumors grown in Swiss mice were...
sensitive to the effects of the deficiency; only solid S-180, however, underwent regression, although E-ca was extruded through ulcerated skin in some of the animals. C57BL/6 mice were particularly sensitive to the toxic effects of the deficiency, and this may account in part for the lack of significant increases of survival time of mice in which Ad-755 had regressed. The inhibition of several ascitic tumors was not associated with prolonged survival of the mice regardless of differences in host sensitivity to the deficiency.

**Table 4**

**Effects of Pyridoxine Deficiency on a Spectrum of Mouse Tumors and Their Hosts**

<table>
<thead>
<tr>
<th>Tumor (host)</th>
<th>No. mice</th>
<th>Period of deficiency prior to implantation (weeks)</th>
<th>Maximum tumor growth inhibition* (per cent)</th>
<th>Maximum tumor regressions (per cent)</th>
<th>Mice dead prior to implantation† (per cent)</th>
<th>Mice dead following implantation‡ (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-180 solid (Ha ICR Swiss)</td>
<td>55</td>
<td>7</td>
<td>61</td>
<td>44</td>
<td>11</td>
<td>56</td>
</tr>
<tr>
<td>S-180 ascitic (Ha ICR Swiss)</td>
<td>50</td>
<td>4</td>
<td>45</td>
<td>0</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>E-ca (Ha ICR Swiss)</td>
<td>18</td>
<td>7</td>
<td>52</td>
<td>0</td>
<td>0</td>
<td>84</td>
</tr>
<tr>
<td>E/2 (Ha ICR Swiss)</td>
<td>50</td>
<td>7</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Ad-755 (C57BL/6)</td>
<td>45</td>
<td>7</td>
<td>53</td>
<td>14</td>
<td>34</td>
<td>90</td>
</tr>
<tr>
<td>L1010 (DBA)</td>
<td>50</td>
<td>7</td>
<td>68</td>
<td>0</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>L4046 (AKR)</td>
<td>50</td>
<td>7</td>
<td>68</td>
<td>0</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>P70429 (CSHI)</td>
<td>50</td>
<td>2</td>
<td>62</td>
<td>0</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

* On the 5th day after implantation (L1210 and L4046), on the 6th day after implantation (E/2), on the 8th day after implantation (S-180 solid), on the 10th day after implantation (S-180 ascitic), on the 14th day after implantation (Ad-755 and P70429) and on the 28th day after implantation (E-ca). Evaluation based on the average diameter (solid tumors) or on cell counts (ascitic tumors).

† At the nutritional condition which gave the maximum inhibition.

‡ Per cent of mice implanted with tumor.

§ Sixteen per cent of the tumors were extruded through ulceration.

**Discussion**

The complete regression of a significant number of Sarcoma 180 tumors grown in pyridoxine-deficient Swiss mice was recently shown in this laboratory (9). This observation is of particular significance in view of the fact that this tumor does not regress spontaneously through resorption. Data obtained in the present study suggest that this phenomenon may occur only as a result of certain tumor-host relationships. In fact, it is of interest that two other tumors transplanted in the same strain of deficient mice did not show any tendency to regress. Thus, a subline of Sarcoma 180, routinely carried in ascitic form, did not regress after either intraperitoneal or subcutaneous inoculation. Similarly, Ehrlich carcinoma (solid) transplanted subcutaneously was inhibited in the deficient animals but did not undergo regression as was seen with solid S-180.

Differences in the sensitivity of different strains to the effects of pyridoxine depletion were apparent (Table 4). Adenocarcinoma 755, grown in pyridoxine-deficient C57BL/6 mice, was inhibited and regressed completely in a number of animals. In this case, however, survival of tumor-free hosts was not longer than that of the controls, owing probably to the relatively high sensitivity of these mice to the effects of the deficiency. By supplementing the deficient diet fed to this strain of mice with low levels of pyridoxine, it may be possible to moderate the severity of the deficiency and to prolong the survival time of the animals so that the occurrence of regressions can be better evaluated.

Pyridoxine depletion of the hosts or treatment with 6-mercaptopurine caused regression of Sarcoma 180 (4, 9). The participation of host defense mechanisms in the induction of these regressions was suggested by their prevention by cortisone (9, 15). Permanent regressions of Sarcoma 180 do not follow the marked inhibition of growth by agents as effective as N-methylformamide (3) or 6-diazo-5-oxo-L-norleucine (5), even though such inhibition is as great as that following treatment with 6-mercaptopurine (4). Treatments that inhibit tumor growth are rarely associated with regression of established tumors. The two phenomena, therefore, are not necessarily dependent upon the same mechanism. The regressions observed in Sarcoma 180 are thus probably dependent upon a particular qualitative effect on the tumor as well as the presence of adequate host...
defence mechanisms.

In this study the growth of seven out of the nine different tumors tested was clearly inhibited in animals fed the pyridoxine-deficient diet starting 2 weeks or more prior to implantation. The failure of the presently known pyridoxine antagonists, such as 4-deoxypyridoxine and isonicotinic acid hydrazide, to produce significant tumor inhibition in animals fed a complete diet (2) may be due to easy reversal of the effects of these antianalabolites by pyridoxine or its congeners. Also, it has been shown that the inhibition of vitamin B6-dependent enzymes by antagonists of pyridoxine is qualitatively different from that observed in B6-deficient animals (6). The pronounced effects of pyridoxine deficiency alone on the growth of various tumors indicate that the depletion of the vitamin impairs the functions dependent upon it more effectively than does treatment with existing pyridoxine antagonists.

The effects that are induced by omitting an essential factor from an otherwise complete diet may indicate important differences in the nutritional requirements of tumor and host. Therefore, the concentrations of vitamin B6 and the activity of certain B6-dependent enzymes are being compared in tumors and other tissues of control and depleted animals. The present studies indicate that an agent capable of interfering with the availability or functions of the vitamin B6 cofactors in a manner duplicating the effects of dietary depletion would selectively inhibit the growth of certain tumors and in some instances induce tumor regression.

SUMMARY

The effects of dietary depletion of pyridoxine on the growth of a limited spectrum of tumors were investigated. This nutritional treatment impaired the growth of six mouse tumors (Adenocarcinoma 755, Leukemia L1210 and L1494, Ehrlich carcinoma ascites clone 2, Ehrlich carcinoma solid, and Plasma-cell tumor 70429) and of one rat tumor (Murphy-Sturm lymphosarcoma) in animals fed a purified deficient diet starting at various periods prior to implantation. Ridgway osteogenic sarcoma and Walker carcinosarcoma 256 were not affected.

Complete regressions comparable to those previously observed with solid Sarcoma 180 under the same conditions were seen only with Adenocarcinoma 755. In this case, however, the host animal was also very sensitive to the toxic effects of the deficiency.

The data obtained support the concept that regression of tumors following therapeutic treat-

ments is dependent upon a particular qualitative effect on the tumors as well as the presence of adequate host defense mechanisms.

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