The Effect of Pyridoxine Deficiency on Mouse Sarcoma 180

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Certain antimetabolites are capable of causing specific effects which can be duplicated in some instances by an acute dietary deficiency of the corresponding metabolite. For example, leukopenia can be induced in experimental animals by feeding a diet lacking folic acid or by treatment with a folic acid antagonist (11, 19). Even though the use of deficient diets seems to be impractical at present as a therapeutic measure, some estimate of the potential effectiveness of new agents may be ascertained by the anti-tumor effects of a specific nutritional deficiency. This concept seems to be particularly applicable in the case of those vitamins for which effective antimetabolites are unavailable.

Several investigators have demonstrated the activity of pyridoxine antagonists, such as 4-deoxypyridoxine and isonicotinic acid hydrazide, against transplanted tumors grown in pyridoxine-deficient animals (6, 10, 13-16). These antagonists, however, did not prove to be effective in animals maintained on a complete diet (6), indicating that their action could be readily prevented by the corresponding vitamin. The retardation of growth of Sarcoma 180 (S-180) and three other transplantable tumors in pyridoxine-deficient animals was initially reported in 1943 (4, 12). Little, if any, further work has been carried out on the effects of prolonged periods of this deficiency alone on tumors. In the present investigation the growth of S-180 has been studied in mice fed a pyridoxine-deficient diet for various periods of time prior to implantation; retardation of growth of the tumor occurred, and, in addition, a consistent incidence of regressions was observed.

MATERIALS AND METHODS

The solid and ascitic forms of S-180 used in these studies were obtained from the Sloan-Kettering Institute through the courtesy of Dr. D. A. Clarke. Female Ha ICR Swiss mice, weighing 18-22 gm., were obtained from the Roswell Park Memorial Institute colony and the Millerton Research Farm, Inc. The mice were kept in shoe box-type cages with sawdust bedding. Pieces of S-180 weighing approximately 5 mg. were uniformly cut by hand and transplanted subcutaneously by trocar into the right axillary region of the animals. Neoplastic growth and body weight were measured on the 8th day after implantation and once a week thereafter. Measurements of the longest diameter of the tumor and of a diameter perpendicular to it were taken through the wet skin by means of vernier calipers, and the average between the two values obtained was considered the index of the size of the tumors.

The ascitic S-180 was inoculated intraperitoneally (1 X 10⁶ cells per mouse). In each experiment total cell counts were performed on five mice per group on the 4th, 7th, and 13th day after inoculation; survival time was evaluated on 5 mice per group.

The purified diet contained 18 per cent casein, 73.8 per cent sucrose, 4 per cent corn oil, 4 per cent salt mixture, 0.5 per cent cod liver oil, 0.2 per cent choline chloride; with the addition per 100 gm. of ration of 1 mg. each of thiamine HCl, riboflavin, and pyridoxine HCl, 4 mg. of niacin, 6 mg. of calcium pantothenate, 15 mg. of inositol, 20 mg. of p-aminobenzoic acid, and 20 μg. each of biotin and folic acid. The deficient diet was prepared similarly, omitting pyridoxine. These diets were prepared weekly, and fresh rations were fed daily ad libitum. Animals were fed the complete purified diet for at least 1 week prior to the feeding of the deficient one, in order to minimize the effect of dietary adjustment on the feeding habits of the mice. Control groups were fed the complete diet for a period equivalent to the longest time that an experimental group was fed the deficient one.

RESULTS

The influence of pyridoxine deficiency on the growth of S-180 was studied in mice fed a pyridoxine-deficient diet for various periods of time prior to implantation; retardation of growth of the tumor occurred, and, in addition, a consistent incidence of regressions was observed.

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RESULTS

The influence of pyridoxine deficiency on the growth of S-180 was studied in mice fed the deficient diet starting 7, 4, or 2 weeks prior to implantation and on the day of implantation. The results obtained are summarized in Table 1. No significant differences were observed between S-180 grown in control mice and in mice fed the deficient diet starting on the day of implantation. The growth of the tumor was inhibited in mice fed the deficient diet for periods of 2 weeks or longer prior to implantation. In the animals fed the deficient diet before implantation of S-180, the growth of most of the tumors stopped during the 2d week, and many individual tumors showed a tendency to diminish in size.
A series of experiments was performed with the specific aim of finding out whether the tumors would actually regress completely. Two of these experiments are summarized in Chart 1. The control tumors grew rapidly, killing their hosts within 5 weeks after implantation. Tumors implanted in the deficient mice, however, regressed after initial growth. It should be pointed out that this is a real regression which takes place by a slow resorption of the tumor. This phenomenon is different from the spontaneous extrusion of the tumor, through ulcerated skin, which occurs in a low percentage of animals bearing S-180. Not all the tumors regressed completely. Some grew slowly and eventually killed their hosts; after an initial partial regression, others resumed their growth. At the 11th week after implantation, however, nine out of twenty mice were surviving in the group fed the deficient diet starting 7 weeks prior to implantation, and sixteen out of twenty were surviving in the group fed this diet starting 2 weeks prior to implantation. All these animals were free of S-180. After all the tumors had regressed and remained nonpalpable for at least 4 weeks, the mice were fed Purina Chow pellets in place of the purified deficient diet. No tumors reappeared during the following 7 weeks. The number of regressions observed in all these experiments are summarized in Chart 2. A distinction was made between partial and complete regressions, the latter including only those tumors which remained nonpalpable for a minimum of 12 weeks after complete resorption. No regressions were observed among 168 tumors grown in mice fed the complete diet. Twenty-two out of 148 tumors regressed in mice fed the deficient diet starting on the day of implantation; however, only seven of these tumors regressed completely. Seventy-five out of

TABLE 1

<table>
<thead>
<tr>
<th>PERIOD OF DEFICIENCY PRIOR TO IMPLANTATION (WEEKS)</th>
<th>NO. MICE</th>
<th>8TH DAY AFTER IMPLANTATION</th>
<th>13TH DAY AFTER IMPLANTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Av. Δ weight* (gm.) (per cent)</td>
<td>Av. tumor diam.* S.D. (mm.)</td>
</tr>
<tr>
<td>DIET</td>
<td></td>
<td>Av. mortality Av. mortality</td>
<td>Av. mortality Av. mortality</td>
</tr>
<tr>
<td>Complete</td>
<td>996</td>
<td>0.4 0.5 9.5±2.9 12</td>
<td>0.5 0.6 9.4±2.3 12</td>
</tr>
<tr>
<td>Pyridoxine-deficient</td>
<td>230</td>
<td>0.4 0.5 9.4±2.3 12</td>
<td>0.5 0.6 9.3±2.2 12</td>
</tr>
<tr>
<td>&quot; 2</td>
<td>188</td>
<td>0.5 0.6 9.5±2.9 12</td>
<td>0.6 0.7 9.4±2.3 12</td>
</tr>
<tr>
<td>&quot; 4</td>
<td>114</td>
<td>0.3 0.4 9.3±2.2 12</td>
<td>0.4 0.5 9.2±2.1 12</td>
</tr>
<tr>
<td>&quot; 7</td>
<td>40</td>
<td>0.6 0.7 9.7±2.3 12</td>
<td>0.6 0.7 9.6±2.2 12</td>
</tr>
</tbody>
</table>

* Average change in body weight from that on day of implantation.
† The deficient diet was given starting on the day of implantation.

Chart 1.—Effect of pyridoxine-deficiency on Sarcoma 180

Chart 2.—Regression of Sarcoma 180 grown in B₆-deficient mice.

Complete regressions: tumors which remained nonpalpable for a minimum of 12 weeks after complete regression, expressed as percentage of total number.

Numbers on top of the bars indicate the number of mice in the groups.
129 tumors regressed in mice fed the deficient diet starting 2 weeks prior to implantation; 47 of these tumors regressed completely. No significant additional increase in the number of complete regressions was evident when the mice were fed the deficient diet for more than 2 weeks prior to implantation. The defect in pyridoxine metabolism responsible for tumor inhibition appears to occur to a maximum extent within a period of 2 weeks.

Pyridoxine or its congeners could prevent both the growth inhibition and the regressions caused by the specific deficiency when supplied in the diet starting on the day of implantation (Table 2). Similarly, in another experiment in which the ascitic form were also studied. The growth of S-180 was inhibited in the deficient mice, as measured by cell counts on the 10th day after inoculation; this inhibition, however, was not of the same magnitude as that observed with the solid tumor (Table 4). Furthermore, the survival time of the host was not prolonged by the deficient diet. These differences between solid and ascitic S-180 are difficult to explain at present.

The results presented in Table 5 show that cortisone had no effect on the growth of S-180, whereas it markedly reduced the number of regressions of the tumors grown in pyridoxine-deficient mice. These results suggest that the

**TABLE 2**

PREVENTION OF THE INHIBITION OF TUMOR GROWTH BY PYRIDOXINE CONGENERS

<table>
<thead>
<tr>
<th>Diet</th>
<th>Vitamin B₆</th>
<th>8TH DAY After Implantation</th>
<th>15TH DAY After Implantation</th>
<th>Regressions at 9TH WEEK After Implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mortality</td>
<td>Inhibition</td>
<td>Mortality</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>Av. weight* (per cent)</td>
<td>Av. tumor diameter ± S.D.</td>
<td>Av. weight* (per cent)</td>
</tr>
<tr>
<td>Complete</td>
<td>20</td>
<td>-0.1 0</td>
<td>10.1±1.4</td>
<td>-4.1 10</td>
</tr>
<tr>
<td>Pyridoxine-deficient†</td>
<td>20</td>
<td>+0.6 0</td>
<td>5.8±1.5</td>
<td>+0.5 0</td>
</tr>
</tbody>
</table>

Pyridoxine† | 18 | +1.0 5 | 8.7±3.3 | -1.6 25 | 12.9±6.6 | 8 0

Pyridoxal† | 20 | +1.2 0 | 9.8±1.2 | -3.1 20 | 14.7±3.4 | 0 0

Pyridoxal-amine† | 8 | +1.0 0 | 9.5±1.0 | -3.0 13 | 13.7±2.2 | 2 0

* Average change in body weight from that on day of implantation.
† The deficient diet was fed for 2 weeks prior to implantation of tumor.
‡ Added to the deficient diet on the day of implantation; 10 mg/kg of diet.

**TABLE 3**

EFFECT OF FOOD RESTRICTION ON THE GROWTH OF SARCOMA 180

<table>
<thead>
<tr>
<th>Feeding</th>
<th>No.</th>
<th>Mortality</th>
<th>Av. tumor diameter ± S.D.</th>
<th>15TH DAY After Implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. weight* (per cent)</td>
<td>Av. tumor diameter ± S.D.</td>
<td>Mortality</td>
<td>Inhibition</td>
</tr>
<tr>
<td>Ad libitum</td>
<td>20</td>
<td>-1.9 1</td>
<td>9.2±1.8</td>
<td>-1.9 1</td>
</tr>
<tr>
<td>Restricted†</td>
<td>20</td>
<td>-0.8 2</td>
<td>7.5±1.8</td>
<td>-2.4 4</td>
</tr>
</tbody>
</table>

* Average change in body weight from that on day of implantation.
† Food sufficient for 1 day was made available to the animals only for 24 hours every 3d day.

**TABLE 4**

EFFECT OF PYRIDOXINE DEFICIENCY ON THE GROWTH OF SARCOMA 180 IN ASCITIC FORM

<table>
<thead>
<tr>
<th>Period of deficiency</th>
<th>15TH DAY After Implantation</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. cell no. X 10⁶ (per S.D. * cent)</td>
<td>Time (days)</td>
</tr>
<tr>
<td>Complete</td>
<td>922±19</td>
<td>16</td>
</tr>
<tr>
<td>Pyridoxine-deficient</td>
<td>697±12</td>
<td>25</td>
</tr>
</tbody>
</table>

* These data represent two combined experiments in each of which 25 mice per group were kept for observation of survival time and five mice per group were used for cell counts.
regressions of the inhibited tumors were brought about by some host defense mechanisms which could be blocked by cortisone. It was of interest to see whether S-180 would show the usual capacity for growth when implanted in mice in which complete regression had been induced by pyridoxine deficiency. These animals were maintained on the deficient diet for 4 weeks following complete regression of their tumors. At this time Purina Chow pellets were fed in place of the deficient diet. The animals were used for this experiment 4–12 weeks thereafter. The results obtained are summarized in Table 6. All the implanted tumors grew as expected in 28 normal control mice, whereas only seven tumors grew in one group of ten mice and nineteen in another group of 29 mice. In both of these groups the rate of growth was very slow, and all the tumors were extruded during the following 7 weeks. This indicates that the animals in which S-180 had regressed have acquired resistance to re-implantation of the same tumor.

**DISCUSSION**

Sarcoma 180 does not ordinarily regress even when treated with compounds which reduce its rate of growth. To date, 6-mercaptopurine and certain related active antimetabolites have been the only agents capable of inducing true regressions of this tumor (7, 8). The consistent number of regressions of S-180 which have been observed in pyridoxine-deficient mice acquires, therefore, even greater significance. The growth of S-180 was not impaired in mice fed restricted amounts of the

**TABLE 5**

| Effect of Cortisone on the Growth of Sarcoma 180 in Pyridoxine-Deficient Mice |
|--------------------------|--------------------------|--------------------------|
|                         | Period of Deficiency | Treatment | Day After End of Treatment | 6th Week After Implantation |
|                         | Prior to Implantation | (mg/kg/day) | No. | Weight* | Mortality (no.) | Weight* | Mortality (no.) | Regressions (no.) |
| Complete                | Saline               | 26         | +1.8 | 0       | 10.4±1.4 | 26         | 0                 |
| Complete                | Cortisone 25†        | 27         | −0.4 | 1       | 8.5±2.8  | 27         | 0                 |
| Pyridoxine-Deficient    | 3 Weeks              | Saline     | 27   | +1.1    | 6.5±0.6  | 17         | 0                 |
|                        | Cortisone 25†        | 28         | −0.1 | 0       | 6.6±2.1  | 19         | 0                 |
| Pyridoxine-Deficient    | 2 Weeks              | Saline     | 40   | +1.0    | 9.9±3.6  | 38         | 0                 |
|                        | Cortisone 25‡        | 40         | −0.4 | 2       | 8.4±2.8  | 35         | 0                 |

* Average change in body weight from that on day of implantation.
† Treatment started on the 1st day after implantation and continued for 7 days.
‡ Treatment started on the 5th day after implantation and continued for 7 days.
§ In this group five tumors regressed, and four were extruded through ulcerated skin.
# In this group no tumor regressed, and two were extruded through ulcerated skin.

**TABLE 6**

| Growth of S-180 in Mice in Which the Tumor Had Previously Regressed |
|--------------------------|--------------------------|--------------------------|
|                         | 8th Day After Implantation | 8th Week After Implantation |
|                         | No. | Av. tumor diam.±S.D. | No. | Takes (per cent) | No. of survivors* |
| GROUP OF MICE           | MIC | (mm.) | (no.) |                |                  |
| Normal control†         | 28  | 9.5±1.1 | 28  | 100  | 4               |
| II‡                     | 10  | 2.7±1.0 | 10  | 65   | 29              |

* All the survivors were without tumors; in the animals in which S-180 had originally taken, the tumors were extruded during the 7 weeks following transplantation.
† Normal control: new mice which had never been implanted with S-180.
‡ Mice which in previous experiments had been fed the pyridoxine-deficient diet starting on the day of implantation.
§ Mice which in previous experiments had been fed the pyridoxine-deficient diet starting 2 weeks prior to implantation. Animals of Group I and II had been fed Purina Chow pellets starting 3 weeks prior to their use in this experiment.
# Three tumors out of seven were too small to be measured by caliper and were assigned a standard average diameter of 1.5 mm.
|| Eight tumors out of nineteen were too small to be measured by caliper and were assigned a standard average diameter of 1.5 mm.
complete synthetic diet, this being an indication that the marked inhibition observed in pyridoxine-deficient animals was not due to any extent to nonspecific dietary restriction. Furthermore, complete reversal of the effects of the deficiency in depleted mice was observed when the diet was supplemented with pyridoxine or any of its congeners.

The profound anti-tumor effects observed in mice fed the deficient diet prior to the implantation of S-180 are in striking contrast to the relatively small effects found in animals fed this diet starting on the day of implantation. Apparently, a certain degree of depletion of the host must occur before altered metabolism is reflected by an impaired growth of the tumor. These observations may be limited to particular situations in which the relationship between the sensitivity of the tumor and of the host to pyridoxine deficiency permits a selective effect of the deficiency against the tumor. For example, although the deficiency was associated with retardation of growth of Adenocarcinoma 755 in C57BL/6 mice, it was not possible to observe a consistent number of regressions of this tumor, since prolonged deficiency was associated with a high incidence of mortality. The presently known pyridoxine antagonists do not seem to evoke the over-all anti-tumor effects observed with pyridoxine deficiency alone. This may be because of qualitative differences between the effects of the antagonists and of the deficiency on specific enzymes, as was observed by Dietrich and Shapiro (9), or may be due to a nonspecific toxicity of these agents.

The incidence of tumor regression caused by 6-mercaptopurine was reduced in animals treated with cortisone (18). Treatment with cortisone did not influence the retardation of growth of S-180 in pyridoxine-deficient mice, but consistently reduced the number of regressions under these experimental conditions. Host defense mechanisms of the immunity type may be responsible for the regression of these tumors. Such a hypothesis, although not consistent with the lowered production of antibodies demonstrated in pyridoxine-deficient animals (2, 3, 17), is supported by the reduced number of takes and the lack of continued growth of S-180 observed when the tumor was implanted in mice in which complete regressions had been caused by pyridoxine deficiency. Resistance to re-implantation has been observed in other experimental conditions (1, 5).

The findings described herein clearly indicate that the study of the anti-tumor effects of a nutritional deficiency may disclose potentialities of new antagonists of the specific metabolite. In this particular case the effects of pyridoxine deficiency on S-180 point out the need for the development of new antimetabolites to reproduce chemotherapeutically the regressions induced by the specific nutritional condition.

SUMMARY

1. Growth of solid S-180 was markedly inhibited in pyridoxine-deficient mice, the inhibition being related to the length of time that the animals were fed the deficient diet prior to implantation. A significant number of tumors regressed and remained nonpalpable for 8–16 weeks. Both inhibition and regression were prevented by the administration to the deficient mice of pyridoxine, pyridoxal, or pyridoxamine, starting on the day of implantation of the tumor.

2. Cortisone markedly reduced the number of regressions of S-180 but did not affect the inhibition of its growth by pyridoxine deficiency. S-180 showed an impaired capacity to take and to grow in mice in which this tumor had previously regressed.

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