Effect of Pyridoxine and Desoxypyridoxine on Rat Fibrosarcoma Grafts*

JOHN B. LOEFER

(Foundation of Applied Research, San Antonio, Texas)

Several reports in the literature on induced and transplanted tumors suggest that vitamin B₆ may be a factor of importance relative to incidence and growth of induced and transplanted tumors. When the Flexner-Jobling carcinoma was implanted in rats partially deficient in pyridoxine, the percentage of takes was lower (66 versus 85 per cent), the number of regressions higher (30 per cent versus none), and the size of the tumors smaller than in control animals (20) on an equivalent caloric intake. Similar results were reported for mice inoculated with a fibroid sarcoma (56 versus 76 per cent takes) or the Yale carcinoma No. 1 (71 versus 92 per cent takes). The incidence of skin tumors induced by methylcholanthrene painting likewise was lower in pyridoxine-deficient animals (36 versus 62 per cent). With diets containing p-dimethylaminoazobenzene the incidence of rat liver tumors paralleled the amount of available pyridoxine, so that when vitamin B₆ in the diet was increased or reduced, tumor incidence was correspondingly increased or reduced (25, 26). Benzpyrene-induced epithelial tumor incidence in mice chronically deficient in pyridoxine was 82 per cent lower than in controls, and this effect was independent of caloric intake, according to Boutwell and Rusch (4). A lowered incidence of benzpyrene-induced epithelial tumors in mice on low B-vitamin rations was observed by Boutwell, Brush, and Rusch (3) and interpreted to be the result of vitamin B₆ deficiency. Other workers (2) reported that if vitamin B₆ was omitted from an otherwise adequate mouse diet, there was a marked decrease in the growth rate of Sarcoma 180.

On the other hand, Morris (27, 28) noted that vitamin B₆ deficiency seemed not to affect growth of spontaneous mammary tumors in C3H mice. Nontumor-bearing B₆-deficient mice were able to survive for 8 weeks, but they lost weight, developed necrotic tails and encrusted ears, lost hair, and became partially paralyzed. The tumor-bearing mice on the same deficient diet, however, exhibited no symptoms of this type, and tumor growth appeared unaffected. Pyridoxine did not stimulate growth of the Rous chicken sarcoma (23), although a positive response was obtained with folic acid, niacinamide, calcium pantothenate, riboflavin, and chol conjugated acid.

Since Woods’s (40) report on growth inhibition by metabolite analogs, other vitamin inhibitors have been used by a number of investigators (35, 41, 42) to demonstrate deficiency diseases in animals. This concept was recently discussed by Greenberg and Schulman (15) in relation to cancer research, and a number of reports have appeared on the use of certain metabolite analogs as tumor growth inhibitors. Stoerk (33) used the pyridoxine analog desoxypyridoxine for experiments on lymphosarcoma 6C3H-ED in hybrid mice (C strain females crossed with C3H males). His experimental animals were kept on a B₆-deficient diet for 3 weeks, and 300 µg. of desoxypyridoxine was added per milliliter of drinking water. Pyridoxine supplemented the diet of control animals. Four weeks after implantation, twelve of fourteen control animals (86 per cent) had tumors, while only two of twelve (17 per cent) on the desoxypyridoxine diet had developed tumors. Tumors of animals on the B₆-deficient desoxypyridoxine diet were much smaller than those of controls.

Other antagonists, e.g., isoriboflavin (35), folic acid analogs (5, 6, 10, 19, 32, 37, 38, and 39),

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purine and pyrimidine analogs (6, 11, 13, 14, 18, 21, 31, and 36) have been used to produce or intensify the effects of certain deficiencies in attempts to control growth of tumors and leukemia.

Our investigations were carried out to determine whether pyridoxine might be a factor influencing implantation of the rat tumor used in this laboratory. A previous study (24) had shown that the percentage of takes could be correlated with age of the host. It was postulated that the phenomenon observed might be attributable to the nutritional status of the host at different ages. A rational approach to the solution of this problem, at least as far as vitamin B6 was concerned, was possible through the use of supplementary pyridoxine and its antagonistic analog desoxypyridoxine.1 The transplantable rat fibrosarcoma previously used was utilized in this study.

They were segregated into groups of 30 and treated during the next 2 weeks as indicated below. Subsequently all received implants of grafts from the same stock, and 20 days later takes were recorded as shown in Table 1. Each of thirty control animals received thirteen daily intraperitoneal injections of 1 ml. distilled water. Another group was given a series of thirteen pyridoxine injections of 100 µg. each in 1 ml. distilled water. Total pyridoxine given per animal amounted to 1,300 µg. Each animal of the third group received intraperitoneally 5 mg. of desoxypyridoxine dissolved in 1 ml. distilled water per day. The total amount given to each animal was 65 mg. As may be seen in Table 1, the highest percentage of takes was observed in the pyridoxine-treated rats.

For series 3 another group of 30 male rats, also 5 weeks old, was used. During a 2-week period, the

| TABLE 1 |
| EFFECT OF PYRIDOXINE AND DESOXYPYRIDOXINE ON A TRANSPLANTED RAT FIBROSARCOMA |

<table>
<thead>
<tr>
<th>SERIES NO.</th>
<th>CONTROLS</th>
<th>PYRIDOXINE-TREATED</th>
<th>DESOXYPYRIDOXINE-TREATED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. rats</td>
<td>No. takes</td>
<td>Per cent</td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Totals and averages:</td>
<td>81</td>
<td>13</td>
<td>16</td>
</tr>
</tbody>
</table>

**METHODS AND RESULTS**

Ninety 5-week-old male rats were used in series 1. All had been raised on a Purina Laboratory Chow diet and had remained on this regimen during the course of the experiment. The animals were randomly segregated into three groups. Thirty rats of the control group each received eight 1-ml. intraperitoneal injections of distilled water during the next 14 days. Each of the 30 animals in the second group received eight intraperitoneal injections of 100 µg. pyridoxine during the 2-week period. Animals of a third group were each given eight 1-ml. injections of distilled water containing 5 mg. of desoxy.pyridoxine per milliliter. After the 2-week treatment period, when the rats were 7 weeks old, implants from an 18-day stock tumor were made to all animals. Thirteen days later, the percentage of takes in each group was recorded (Table 1).

Ninety male animals comparable in age and weight to those of series 1 were used in series 2. 30 control animals each received eleven injections of 1 ml. distilled water. Sixteen days after the first injection, and following the tumor implants, four more were administered. Each of another 30 rats received eleven intraperitoneal injections of 100 µg. of pyridoxine before, and four more after, receiving tumor grafts, for a total of 1,500 µg. per rat. The third group received an equal number of 10 mg. desoxy.pyridoxine injections, for a total of 150 mg. per rat. Tumors were palpated at 15 days, and the record is shown in Table 1.

Animals that received desoxy.pyridoxine gained only 40 per cent in weight, as compared to controls and pyridoxine-treated rats which gained 46 per cent. Of those that received desoxy.pyridoxine, only a small number developed minor cutaneous lesions on the tail, paws, snout, and ears, but none showed ventral curling of the distal portion of the tail.

Pyridoxine-treated animals exhibited a higher average percentage of takes (40 per cent) than either controls (16 per cent) or desoxy.pyridoxine-treated rats (11 per cent). The difference in the latter two figures is not considered significant.

Another experiment was carried out with hamsters to determine whether pyridoxine-deficiency

1 Merck's pyridoxine (2-methyl-5-hydroxy-4,5-dihydroxy-methylpyridine) and desoxy.pyridoxine (2,4-dimethyl-5-hydroxy-5-hydroxymethylpyridine hydrochloride) were used. The latter was furnished through the courtesy of Dr. Augustus Gibson.
symptoms could be established in these animals by administering desoxypyridoxine while they were kept on a diet of Purina Laboratory Chow. Two age groups with twenty hamsters in each were used. One group was 5 weeks and the other 10 weeks old. Ten animals of each age group were used for controls and ten for test animals. The test animals received a total of 190 mg of desoxypyridoxine, given in 5-mg. intraperitoneal injections either daily or at intervals of not more than 3 days, over a period of 59 days (= 2.3 mg per day average). There were no significant weight differences in either the 5- or 10-week test animals, as compared to their controls at the end of the test period, nor were any of the characteristic symptoms of vitamin B₆ deficiency observed such as described by others (29, 30).

**DISCUSSION**

Since supplementary pyridoxine resulted in a higher percentage of takes, it is of interest to consider the minimum vitamin B₆ requirement of the rat. Most estimates (7, 8, 22) agree that the amount needed daily is about 10 μg. For thehamster 3 μg. per day was sufficient to prevent the acrodynia-like dermatitis which characterizes vitamin B₆ deficiency (29). There is some evidence that rats on a Purina Laboratory Chow diet may require a considerable amount of the daily vitamin B₆ requirement appears to be obtained from other sources; possibly it is provided by the bacterial flora.

Even if this explanation were correct, the question still remains why desoxypyridoxine administered in quantities up to 10,000 μg. per day for 15 days, as in series 3, did not cause deficiency symptoms in most of the rats. The ratio of desoxypyridoxine to pyridoxine over this period, on the basis of the daily subsistence requirement of B₆ was approximately 1,000:1. Emerson (9) was unable to demonstrate deficiency symptoms by omitting pyridoxine from a rat diet, although when desoxypyridoxine was added, typical acrodynia developed after 55 days if the ratio of analog to vitamin was 50:1 or greater. It is possible, of course, that the period of administration of desoxypyridoxine in our experiments was not long enough for deficiency symptoms to develop in more than a few cases. Or, possibly, the tumor-bearing animals differed from normal rats, as the experiments of Morris (27) suggest. We did not treat nontumor-bearing rats with desoxypyridoxine, hence have no data bearing on this point. Another possible explanation for failure of deficiency symptoms to appear in many rats is that desoxypyridoxine may not have acted as an inhibitor to pyridoxal or pyridoxamine, the other forms of vitamin B₆. Perhaps the action is more involved, for, not only does this vitamin function in normal metabolism (1), but, according to the report of Keresztesy et al. (17), it may act indirectly by counteracting inositol inhibition of Sarcoma 180. Inositol inhibition of this tumor was also counteracted by p-aminobenzoic acid and other compounds such as thiamin, niacinamide, and leukopterin.

While instances of success in controlling tumor development were cited in the introduction, it might be worth pointing out that certain other reports indicate failure to control tumor growth by the use of desoxypyridoxine. Gregoire (16), for example, reported that lymphosarcoma development in young rats was delayed by a vitamin B₆ deficiency induced by certain quantities of desoxy- pyridoxine, but use of larger doses gave inconclusive results. Gellhorn and Jones (12), in a report on six clinical cases of which three were leukemia and three lymphosarcoma, stated there was no evidence that restriction of pyridoxine in the diet, together with administration of desoxypyridoxine for periods up to 2 weeks, had any therapeutic effect on lymphosarcoma or acute leukemia.

**SUMMARY**

The incidence of fibrosarcomas, following subcutaneous grafting into 7-week-old control rats living on a Purina Laboratory Chow diet, was compared to that in rats which received supplements of pyridoxine and in others that were given desoxypyridoxine; 243 animals were used.

Pyridoxine supplements per rat totaled as much as 1,500 μg. over a 2-week period before implantation and up to 400 μg. following grafting. Desoxypyridoxine injections totaled as much as 110 mg. per rat preceding tumor injections and up to 40 mg. subsequently.

Tumor takes after 13–20 days in pyridoxine-treated rats averaged 40 per cent, as compared to takes of 16 and 11 per cent, respectively, in controls and desoxypyridoxine-treated animals. Pyridoxine deficiency symptoms were noted in only a few rats bearing tumors in the desoxypyridoxine-treated group.

Although this analog was also administered in relatively large amounts to nontumor-bearing hamsters on an identical basic diet over a period of 59 days, no vitamin B₆ deficiency symptoms were observed.

*H. L. Wilcke, personal communication, 1940.
ACKNOWLEDGMENT

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REFERENCES

28. NUTS, H. P. Vitamin Requirements of the Mouse. Vitamins and Hormones, 6:175-95.
41. WOOLLEY, D. W. Production of Nicotinic Acid Deficiency with 2-Acetylpyridine, the Ketone Analogue of Nicotinic Acid. J. Biol. Chem., 157:655-9, 1945.
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John B. Loefer

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