The Effect of Nitrofurazone on Growth of Fibrosarcoma in Mice

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Nitrofurazone N.N.R., 5-nitro-2-furaldehyde semicarbazone (furacin), has been found to be effective in controlling the bacterial flora of infected wounds (10-12). Dodd (2) reported that furacin was active in vitro, and in some cases in vivo, against a large variety of gram-positive and gram-negative bacteria. Cramer (1) showed that furacin poised the oxidation-reduction potential of bacteria and suggested that it might interfere with the enzymes involved in hydrogen transport. Green (3) observed that furacin inhibited the oxidation of glucose and pyruvate in the Warburg respirometer, when bacteria were used as the source of enzymes.

Since furacin appeared to inhibit several enzyme systems of bacteria, its action on tumor growth was also investigated. In a preliminary report, Green and Friedgood (4) found that furacin retarded the growth of a transplanted fibrosarcoma in mice. In this paper, further observations of the effects of furacin on the growth and histology of this tumor will be described.

MATERIALS AND METHODS

Ninety adult inbred C3H mice of the Andervont subline were used. Equal amounts of mouse sarcoma 5-13 were transplanted into the right axillary space. Furacin crystals, finely ground to a powder in a mortar, were suspended in peanut oil at a concentration of 200 mg/ml. This suspension was injected subcutaneously into the lumbar dorsal region at a site far removed from the region of the tumor implantation. The animals were divided into six groups and treated with furacin (20 mg. in 0.1 ml. oil) according to the following treatment schedules:

Group I.—One dose injected 1 week after implantation of the tumor.

Group II.—Injected 3 days prior to transplantation, followed by another injection 1 week after transplantation.

Group III.—Injected 3 days prior to transplantation, 1 week after transplantation, and again 2 weeks after transplantation.

Group IV.—Injected 5 days before transplantation, again at the time of transplantation, and thereafter every 5 days until 25 days had elapsed, making a total of 140 mg. of furacin per mouse.

Group V.—Untreated controls.

Group VI.—Controls treated with five doses of 0.1 ml. of peanut oil at intervals of 5 days after transplantation.

The tumors were palpated daily, to note the amount of growth. The mice were weighed periodically, to determine any abnormal losses in weight. Necropsies were made at the time of death of the animals, and microscopic sections were prepared from the tumor, kidney, liver, adrenals, and gonads. All animals were fed Purina Fox Chow and housed in wire cages.

RESULTS

Table 1 compares the survival time of the tumor-bearing animals treated with furacin with that of the untreated controls. All treated animals showed an increased longevity.

The effect of furacin given only after tumor implantation is seen in the results with Group I. When Furacin was given before as well as after implantation, the greatest effects on longevity were observed. In Groups II, III, and IV, the average life span increased from 34 days to 40 days. The lower limit of longevity in each case was at least

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30 days, when furacin was also given before implantation. This result may be attributed to the 20 mg. of furacin given before transplantation. However, doses of furacin given after transplantation also had an effect, in raising the upper limit of the life span from 34 days in Group I to the maximum of 49 days in Group IV. There is not much advantage, in terms of longevity, in increasing the total dose of furacin from 60 mg. to 140 mg.

The effect of furacin was not influenced by the peanut oil in which it was suspended. The rate of tumor growth and the life span of the mice treated with peanut oil was approximately the same as that of the untreated control group.

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Total furacin injected (mg.)</th>
<th>Average survival (days)</th>
<th>Range</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>20</td>
<td>29</td>
<td>22–34</td>
<td>3.6</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>40</td>
<td>34</td>
<td>30–42</td>
<td>3.6</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>60</td>
<td>37</td>
<td>30–47</td>
<td>3.7</td>
</tr>
<tr>
<td>IV</td>
<td>30</td>
<td>140</td>
<td>40</td>
<td>31–49</td>
<td>3.7</td>
</tr>
<tr>
<td>V</td>
<td>15</td>
<td>Controls</td>
<td>21</td>
<td>18–25</td>
<td>2.6</td>
</tr>
<tr>
<td>VI</td>
<td>15</td>
<td>Controls plus peanut oil</td>
<td>22</td>
<td>19–27</td>
<td>3.4</td>
</tr>
</tbody>
</table>

* Details in the text.

Although furacin exhibited a definite inhibitory effect on the growth of the tumor, eventually all the animals died as a result of the malignancy. In the control series, the tumor was usually palpable within 5 days following transplantation, while in the furacin-treated mice the tumor transplant was not palpable for at least 2 weeks and grew at a correspondingly slower rate. There was no evidence of metastasis in any of the animals. The sarcoma, however, did infiltrate the surrounding tissues and the thoracic cage.

Histologic studies revealed a striking effect of furacin on the morphology of the sarcoma in treated animals. The microscopic picture of the untreated tumor was typical of a highly cellular fibrosarcoma. In sections of the furacin-treated tumor tissue there was evidence of cellular degeneration with pyknotic nuclei and a more granular cytoplasm. The tissue took an eosinophilic stain, and mitosis was greatly decreased as compared to that in the untreated controls. The microscopic differences correspond to the gross differences observed in the rate of growth.

There was no evidence of parenchymal damage to the liver or kidney. The adrenal gland, however, was hypertrophied in the furacin-treated animals. The testes and ovaries were atrophic with decreased gametogenic activity.

**DISCUSSION**

Relatively little is known regarding tumor chemotherapy. The "metabolite-antagonism" approach, which has been extensively applied in bacterial chemotherapy, has also been suggested for the treatment of tumors (5). Vitamin (9, 13) and purine (7) analogs have been tested against tumors.

Both the growth experiments and the histologic changes described above indicate that furacin may selectively inhibit the growth of neoplastic tissue. While a definite mechanism of action cannot be advanced at the present time, two possibilities are suggested. The first concerns the possible inhibition of glucose or pyruvate metabolism of the tumor by furacin.

Studies on bacterial metabolism with furacin (3) have shown that it inhibits the enzymes involved in the dissimilation of glucose and pyruvate. Recent experiments have shown that several nitrofurans likewise inhibit glucose dissimilation. The homologous non-nitrofurans are inactive in this respect.

Since members of the vitamin B complex in coenzyme form are involved in glucose and pyruvate metabolism, the following experiments not previously described may be of interest. Eight adult, albino Wistar rats were injected subcutaneously with two large doses of furacin suspended in peanut oil—200 mg. initially and the same dose again after 1 week. At the end of 2 weeks, characteristic symptoms of multiple vitamin deficiencies were observed, such as porphyrin-whiskers, rusty fur, scaly skin and feet, weakening of the hind legs, diarrhea, cheilosis, and conjunctivitis. When the administration of furacin was terminated and the animals were fed on a full diet fortified with vitamins, they returned to their original healthy state of nutrition. While no symptoms of vitamin deficiency were observed under the conditions of the tumor therapy experiments, these experiments nevertheless indicate the possibility that furacin may interfere with the carbohydrate metabolism of the tumors. However, before coming to this conclusion, appropriate experiments with the treated tumors will have to be performed.

The role of the adrenal cortex suggests another possible explanation for the mode of action of furacin on tumor growth. Lewis, Apteckman, and King (8) have demonstrated the retardation of tumors by mincing tumor grafts with adrenal

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Green, M. N.; Heath, E. C.; and Goldberg, H. Unpublished data.
gland tissue alone. Complete inhibition of growth of a transplanted rhabdomyosarcoma in C3H mice treated with daily injections of 1.0 mg. of cortisone has been reported by Higgins, Woods, and Bennett (6). Since hypertrophy of the adrenal cortex was observed in the treated animals, furacin may exert its effect on tumor growth indirectly through the adrenal gland. Investigations now being conducted on adrenalectomized mice should clarify this possibility. The observed atrophy of the testes and ovaries following prolonged furacin therapy may also be related to the enlargement of the adrenal cortex.

SUMMARY
Furacin suspended in peanut oil, injected subcutaneously, retarded the growth of fibrosarcoma S-13 in C3H mice of the Andervont subline. The life span of the mice was approximately doubled by this treatment (from 21 to 40 days). Microscopic examination of the furacin-treated tumors revealed increased cellular degeneration, with lessened mitotic activity. Hypertrophy of the adrenal cortex and atrophy of the testes and ovaries have also been observed in the furacin-treated animals.

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REFERENCES
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