The Effect of Heptyl Aldehyde-Sodium Bisulfite on Spontaneous Tumors of the Mammary Gland in Mice*

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Data have been presented (17) which indicate that an indirect correlation exists between the growth rate and fate of spontaneous tumors and the amount of heptyl aldehyde-sodium bisulfite compound injected. This finding applied to doses ranging from 2 mgm. to 6 mgm. per diem. It was further stated that experiments dealing with tumor-bearing mice receiving larger doses were under investigation. The present report includes the complete data for the series of animals injected with graded doses from 2 to 13 mgm. per diem.

**Materials and Methods**

All mice in this experiment were fed a diet of NURISHMIX. Only A strain multiparous mice with spontaneous tumors of the mammary gland were used. This selection was made since the female mice of the A strain develop tumors having the highest degree of malignancy of any of the pure strains of mice in our laboratory. The criteria of malignancy used were: (a) the growth rate of the tumors as indicated by the average size measured in the longest linear diameter, (b) the number of multiple primary tumors, (c) the number and size of pulmonary metastases, and (d) the survival time, in days, of the tumor-bearing mouse after the discovery of its tumor. Two hundred and fifty mice were used as experimental animals with heptyl aldehyde-sodium bisulfite; 120 mice of approximately similar age and size of initial tumors were used as controls.

A daily subcutaneous injection of an aqueous solution of heptyl aldehyde-sodium bisulfite 1 with the indicated dose was given following the weighing of the mouse and the examination of its physical appearance. A particular mouse received either its full dose or none at all. No attempt was made to vary the daily dose with the body weight of the mouse. Each injection in every series consisted of 0.2 cc. in volume. Solutions were made up daily so that 0.2 cc. would contain the desired daily dose for the particular series. In the higher doses employed severe local ulceration occurred and this interfered with subsequent treatment. The highest daily dose used (13 mgm.) is approximately one-half the minimal lethal dose for mice under the laboratory conditions of the present investigation.

The tumors in all mice were measured by vernier calipers in the two greatest diameters at weekly intervals. For the computation of growth rate in this study only the measurement of longest linear diameter was used. This procedure was followed since a too accurate calculation of the "volume" of a tumor is not warranted for one or more of several reasons. These are: (a) the variable thickness of skin over a tumor cannot be accurately determined; (b) the relative amount of stroma and cancer tissue from time to time is variable; (c) the relative amount of necrosis and hemorrhage is also variable, although both processes usually increase with the size of the tumor, but not always; and (d) the presence of liquefaction and subsequent cystic areas interferes with the estimate of amount of the actual living cancer tissue.

**Results**

With the divided daily dose, many mice received a total of more than 1,000 mgm. of heptyl aldehyde-sodium bisulfite during their survival period. The maximal total dose that any mouse received was 1,614 mgm. (in the 6 mgm. daily series). This mouse (No. 131998) was injected with 6 mgm. 269 times and survived 305 days following the discovery of her tumor. The longest survival time of a mouse after the discovery of its tumor was 439 days, at which time the mouse (No. 141302) was 710 days old. She had received 775 mgm. in a total of 129 injec-
tions. At death there was no evidence of the original tumor or any other tumor. Two of the mice, Nos. 1433447 and 144262, are still living, free of any mammary tumor at 309 and 218 days, following the regression of their spontaneous tumors.

The data obtained in the completed series of doses of heptyl aldehyde-sodium bisulfite ranging from 2 mgm. to 13 mgm. per diem, are given in Table I and Fig. 1. The degree of deviation from a measure of average or control malignancy obtained in this graded series of doses with the same drug is given in Fig. 1. This measure of malignancy was a composite estimate based upon the following criteria, (a) growth rate of the tumors, (b) survival time of the tumor-bearing mouse, (c) number and size of lung metastases, (d) shape of tumors as an indication of monocolicentric or polycentric growth, (e) number of tumors in all series of mice treated with doses between 2 and 11 mgm. per diem. In mice of the 12 mgm. per diem series a mild degree of stimulation was obtained as indicated, especially in the polycentric growth of tumors. Many of the tumors in this series showed no evidence of liquefaction and the tumors became mulberry in shape.

**DISCUSSION**

A series of papers (12-17) dealing with the effects of heptyl aldehyde and its derivatives has been published. The essential feature of this effect on spontaneous tumors of the mammary gland in mice is

### Table 1: Summary of Data

<table>
<thead>
<tr>
<th>Heptyl aldehyde, in mgm.</th>
<th>Number of mice</th>
<th>Average age in days at death</th>
<th>Survival time in days</th>
<th>Average or control malignancy obtained in this graded series of doses</th>
<th>Number with metastases</th>
<th>Number with multiple metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5</td>
<td>435.5</td>
<td>69.6</td>
<td>69.6</td>
<td>504.8</td>
<td>504.8</td>
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<tr>
<td>3</td>
<td>5</td>
<td>414.8</td>
<td>72.4</td>
<td>72.4</td>
<td>487.2</td>
<td>487.2</td>
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<td>4</td>
<td>15</td>
<td>390.6</td>
<td>66.6</td>
<td>66.6</td>
<td>457.5</td>
<td>457.5</td>
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<tr>
<td>Total—2, 3, 4 mgm. series</td>
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<td>413.5</td>
<td>69.5</td>
<td>69.5</td>
<td>483.2</td>
<td>483.2</td>
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<tr>
<td>5</td>
<td>25</td>
<td>448.6</td>
<td>55.3</td>
<td>55.3</td>
<td>503.6</td>
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<td>6</td>
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<td>413.5</td>
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<td>83.9</td>
<td>491.4</td>
<td>491.4</td>
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<td>7</td>
<td>25</td>
<td>419.6</td>
<td>65.9</td>
<td>65.9</td>
<td>497.4</td>
<td>497.4</td>
</tr>
<tr>
<td>Total—5, 6, 7 mgm. series</td>
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<td>427.2</td>
<td>64.8</td>
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<td>494.8</td>
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<td>73.5</td>
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<td>9</td>
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<td>404.8</td>
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<td>487.8</td>
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<td>Total—8, 9, 10 mgm. series</td>
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<td>457.9</td>
<td>76.0</td>
<td>76.0</td>
<td>503.9</td>
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<td>11</td>
<td>25</td>
<td>393.4</td>
<td>82.8</td>
<td>82.8</td>
<td>477.8</td>
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<td>12</td>
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<td>76.5</td>
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<td>62.9</td>
<td>62.9</td>
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<td>496.7</td>
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<tr>
<td>Total—11, 12, 13 mgm. series</td>
<td>75</td>
<td>459.7</td>
<td>74.1</td>
<td>74.1</td>
<td>505.5</td>
<td>505.5</td>
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<td>Total Controls</td>
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<td>71.6</td>
<td>495.9</td>
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<tr>
<td>Controls</td>
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<td>349.1</td>
<td>59.2</td>
<td>59.2</td>
<td>409.0</td>
<td>409.0</td>
</tr>
</tbody>
</table>

This table presents data on (I) number of mice in each series, (II) average age of the mice at the time their spontaneous tumors were discovered, the start of treatment, (III) survival time—the average number of days the mice lived after the discovery of their tumors, (IV) survival time of mice still showing some cancerous tissue at death, (V) the average age of death for both groups—some showing tumors and some free of tumor at death, (VI) average age at death of those mice showing some cancerous tissue at death, (VII) number of mice showing multiple tumors during the extent of the experiment, (VIII) number of mice showing regression of tumors, (IX) number of mice showing pulmonary metastases, (X) percentage of mice showing multiple tumors during the extent of the experiment, (XI) the number of mice showing regression of tumors under treatment, (XII) percentage of mice showing regression of tumors, (XIII) number of mice showing cystic areas capable of being drained by mild suction and (XIV) percentage of mice showing "liquefied" tumors. Composite data for (a) 2, 3, and 4 mgm. per diem, (b) 5, 6, and 7 mgm. per diem, (c) 8, 9, and 10 mgm. per diem, and (d) 11, 12, and 13 mgm. per diem are also given.

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This process of liquefaction has been so extensive at times that drainage by sterile puncture and mild suction has been possible. The present investigation indicates that the extent of liquefaction of spontaneous tumors is determined by the level at which the daily administration of subcutaneous injections of an aqueous solution of heptyl aldehyde-sodium bisulfite has been kept. The maximum degree of liquefaction, 58.6 per cent in the tumors of all treated mice, was obtained in the composite series of 5, 6, and 7 mgm. per diem. The maximum number of instances of complete regression of tumors under treatment (10.6 per cent) was obtained in the composite series of 8, 9, and 10 mgm. per diem. The lowest percentage of pulmonary metastases, (IX) number of mice showing multiple tumors during the extent of the experiment, (X) percentage of mice showing multiple tumors, (XI) the number of mice showing regression of tumors under treatment, (XII) percentage of mice showing regression of tumors, (XIII) number of mice showing cystic areas capable of being drained by mild suction and (XIV) percentage of mice showing "liquefied" tumors. Composite data for (a) 2, 3, and 4 mgm. per diem, (b) 5, 6, and 7 mgm. per diem, (c) 8, 9, and 10 mgm. per diem, and (d) 11, 12, and 13 mgm. per diem are also given.
metastases (4.0 per cent) was obtained in the composite series of 2, 3, and 4 mgm. per diem. In the subsequent composite series there was a progressive increase in the number of pulmonary metastases, 4 per cent in the 2, 3, 4 mgm. series; 18.6 per cent in the 5, 6, and 7 mgm. series; 28 per cent in the 8, 9, and 10 mgm. series; and 33.4 per cent in the 11, 12, and 13 mgm. series. In all cases, however, the percentage of pulmonary metastases was lower than it was in the controls (48.2 per cent). The number of mice showing multiple tumors did not seem to be affected by the experimental procedure of the subcutaneous injection of an aqueous solution of heptyl aldehyde-sodium bisulfite.

Garai (8) has recently reported similar liquefactive processes in spontaneous tumors of mice following the injection of the same drug. He points out that this liquid contains viable tumor cells. We have found that the injection of this liquid into mice belonging to the same strain that gave rise to the spontaneous mammary carcinoma results in a transplantable mammary carcinoma (strain-limited response only).

Garai concludes, however, that the destructive action upon the kidneys and liver exerted by repeated intraperitoneal injections of heptyl aldehyde-sodium bisulfite far outweighs its effect upon the growth and fate of spontaneous tumors. Our findings are not in complete agreement with this conclusion. We have observed the following: (a) a considerable number of control mice with spontaneous tumors show kidney damage; (b) many normal mice without tumors at the age at which spontaneous tumors arise show a high degree of renal disturbance, and (c) the too rapid breakdown of any tissue in mice by x-ray or by any other means is almost always accompanied by renal damage. Since the process of liquefaction in spontaneous tumors is one apparently of breakdown of tissue, it is not unreasonable to conclude that some renal damage should be ascribed to this process rather than exclusively to the heptyl aldehyde-sodium bisulfite itself. Furthermore, we have observed that normal mice without spontaneous tumors can be kept alive in apparently normal health while receiving daily injections of the drug in amounts that will bring about liquefactive processes in neoplastic tissue. These injections did not have any obvious damaging effect on surrounding or internal normal tissues. In view of these conflicting opinions, further work upon the toxic effect of this drug in normal mice free of spontaneous tumors should be done before the final evaluation of the data can be made.

The complicating features of the use of heptyl aldehyde-sodium bisulfite on tumor-bearing mice are: (a) the occasional production of local skin ulcerations with doses of more than 8 mgm. per diem, (b) mild, transitory, unusual irritability, unprovoked squealing and jumping with the larger doses, (c) many mice die of a secondary hemorrhage from the surface of the "liquefying" tumor, (d) secondary infections of liquefying tumors are of frequent occurrence, and (e) liver and renal damage aggravated, perhaps by the too rapid breakdown of neoplastic tissue. Many of these disadvantages may eventually be removed by further research.

It is certain that some effect has been produced on spontaneous tumors of mice by the injection of material at a site remote from the tumor. The observation should warrant further investigation. Further discussion on the use of heptyl aldehyde and its derivatives on experimental cancer by many investigators (1-17) will be forthcoming in a review article.

**Summary and Conclusions**

1. Spontaneous carcinoma of the mammary gland in mice may be influenced by the injection of an aqueous solution of heptyl aldehyde-sodium bisulfite at sites remote from the tumor.
2. This effect may be altered by the amount of the drug administered per diem.
3. There is a graded sequence of inhibition of spontaneous tumors as measured by the criteria employed in this investigation between 2 mgm. per diem and 6 mgm. per diem.

4. There is a reverse sequence of inhibition on tumors between 6 mgm. per diem and 11 mgm. per diem.

5. A daily dose of 12 mgm. per diem actually mildly stimulates spontaneous tumors, especially in polycentric growth.

6. The maximal degree of inhibition of tumors was obtained with 6 mgm. per diem.

7. The maximum number of instances of complete regression of tumors under treatment was 10.6 per cent. This occurred in composite series of 5, 6, and 7 mgm. per diem.

8. The number and size of pulmonary metastases is indirectly proportional to the amount of the drug administered per diem; the minimal number (4.0 per cent of total treated mice) was obtained with 2, 3, and 4 mgm. per diem; the maximal number (33.4 per cent of total treated mice) was obtained with 11, 12, and 13 mgm. per diem.

9. The maximal degree of liquefaction, 58.6 per cent, was obtained with 5, 6, and 7 mgm. per diem. With doses of 10 mgm. and more per diem liquefaction occurred infrequently.

REFERENCES


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