Induction of Tumors in Mice with the Herbicide Succinic Acid 2,2-Dimethylhydrazide

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SUMMARY
A solution of 2% succinic acid 2,2-dimethylhydrazide was given continuously in the drinking water of 6-week-old randomly bred albino mice for the remainder of their lives. The treatment gave rise to tumors of blood vessels, lungs, and kidneys. The tumor incidences in these tissues in the controls were 6, 18, and 0%, whereas in the treated groups the corresponding tumor incidences were 73, 73, and 5%. Light microscopic examination revealed typical angiomata and angiosarcomas of blood vessels, adenomas and adenosarcomas of lungs, and adenomas of kidneys.

The study thus demonstrates the tumorigenicity of the herbicide, succinic acid 2,2-dimethylhydrazide. Since the residues of this chemical occur in fruit, the human population is exposed to it. The environmental implication of this finding and the fact that the hydrazines as a class have umorigenic properties are discussed.

INTRODUCTION
SADH2 is a plant growth regulator, which may be applied effectively over a wide range of concentrations as a foliar spray in water, usually at the blossoming period and thereafter. The compound hastens the ripening of fruits and vegetables, including peaches, nectarines, tomatoes, Brussels sprouts, cherries, grapes, apples, etc. (1, 23). It is a stable chemical, produced and used in substantial quantities in the United States. SADH residues are found in fruit and vegetables sprouts, cherries, grapes, apples, etc. (1, 23). It is a stable chemical, produced and used in substantial quantities in the United States. SADH residues are found in fruit, the human population is exposed to it. The environmental implication of this finding and the fact that the hydrazines as a class have umorigenic properties are discussed.

1 This work was supported by USPHS Contract 1 CP33278 from the National Cancer Institute, NIH. This study was presented in part at the meeting of the American Association of Pathologists, Toronto, Ontario, Canada, on March 13, 1977 (B. Toth and A. Tompa. Tumor Induction with Succinic Acid 2,2-Dimethylhydrazide, a Plant Growth Regulator. Am. J. Pathol., 6 Part 2: 25a-26a, 1977).

2 The abbreviation used is SADH, succinic acid 2,2-dimethylhydrazide.

3 The study thus demonstrates the tumorigenicity of the herbicide, succinic acid 2,2-dimethylhydrazide. Since the residues of this chemical occur in fruit, the human population is exposed to it. The environmental implication of this finding and the fact that the hydrazines as a class have umorigenic properties are discussed.

This report records the tumorigenicity of SADH administered continuously in drinking water to randomly bred Swiss albino mice at the maximum tolerated dose for life.

MATERIALS AND METHODS
Swiss albino mice from the colony randomly bred by us since 1951 were used. They were housed in plastic cages with granular cellulose bedding, were separated according to sex in groups of 10, and were given Wayne Lab-Blox diet in regular pellets (Allied Mills, Inc., Chicago, Ill.) and tap water or the chemical solution ad libitum as described below.

The chemical used was SADH [N-dimethylamino]succinamic acid, butanedioic acid mono[2,2-dimethylhydrazide]) (Chart 1). The SADH (M. W. 160.17, m. p. 162-164°C, more than 99% pure and containing 0.002% 1,1-dimethylhydrazine as impurity) was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis. After 48 hr standing at room temperature, the 2% SADH solution that was used for the chronic experiment contained 0.0013% 1,1-dimethylhydrazine.

Toxicity studies were carried out with SADH prior to the chronic experiments. Seven dose levels of SADH (4, 2, 1, 0.5, 0.25, 0.125, and 0.0625%) were administered in the drinking water for 35 days to Swiss mice. When 4 parameters, (survival rates, body weights, chemical consumption figures, and histological changes) were taken into account, the 2% dose level was found to be suitable for the lifelong treatment. This toxicity technique was developed in this laboratory (15).

The solutions were prepared 3 times/week, and the total consumption of water containing SADH was measured at the same intervals during the treatment period. The solutions were contained in brown bottles because of the light sensitivity of the chemical. The chronic experiment contained 0.0013% 1,1-dimethylhydrazine.

The solutions were prepared 3 times/week, and the total consumption of water containing SADH was measured at the same intervals during the treatment period. The solutions were contained in brown bottles because of the light sensitivity of the chemical. The chronic experimental groups were the following.

Group 1. SADH was dissolved in the drinking water as a 2% solution and was given for the life-span of 50 female and 50 male mice that were 6 weeks (42 days) old at the beginning of the experiment. The average daily consumption of water containing SADH per animal was 6.7 ml for the females and 8.5 ml for the males. Therefore, the average daily intake of SADH was 134 mg for a female and 170 mg for a male.

Group 2. As an untreated control, 100 female and 100 male mice were kept and observed from weaning time (5 weeks old).

The experimental and control animals were carefully...
checked and weighed at weekly intervals, and the gross pathological changes were recorded. The animals either were allowed to die or were killed with ether when found in poor condition. Complete necropsies were performed on all animals. All organs were examined macroscopically and were fixed in 10% buffered formalin. Histological studies were done on the liver, spleen, kidney, bladder, thyroid, heart, pancreas, testis, brain, nasal turbinale, and at least 4 lobes of the lungs of each mouse, as well as on those organs showing gross pathological changes. Sections from these tissues were stained routinely with hematoxylin and eosin.

RESULTS

The survival rates after weaning are shown in Table 1. The data demonstrate that the treatment significantly shortened the survival time compared with the untreated controls. The number and percentages of animals with tumors and their age at death (latent periods) are summarized in Table 2. The 3 statistically significant neoplasms were found in blood vessels, lungs, and kidneys and are described in detail below.

Blood Vessel Tumors. Of treated females, 36 (72%) developed vascular neoplasms. Of these, 26 mice had angiosarcomas and 10 had angiomas. The tissue distribution of angiosarcomas was: liver, 25; fat, 3; muscle, 2; and pancreas, 1. The angiomas were observed in liver (9 mice) and uterus (1 mouse). In treated males, 37 (74%) developed blood vessel tumors. Of these, 34 mice had angiosarcomas and 3 had angiomas. The tissue distribution of angiosarcomas was: liver, 34; lungs, 4; fat, 4; and lymph nodes, 4. All the angiomas occurred in the liver.

Macrosopically and histologically, all observed lesions were similar to those described earlier (19, 22).

Lung Tumors. Of treated females, 37 (74%) developed 164 tumors in this organ. Of these, 25 mice had 70 adenomas, 2 0 H
\[ \text{Chart 1. Chemical structure of SADH.} \]

mice had 2 adenocarcinomas, and 10 mice had 71 adenomas and 21 adenocarcinomas. In treated males, 36 (72%) developed 93 neoplasms in the lungs. Of these, 26 mice had 59 adenomas, 2 mice had 3 adenocarcinomas, and 8 mice had 20 adenomas and 11 adenocarcinomas.

Grossly and histologically, these tumors were similar to those described earlier by workers in this laboratory (18, 21).

Kidney Tumors. Of treated males, 5 (10%) developed 6 tumors in this organ. They were classified as benign adenomas.

Histopathologically, the kidney lesions were similar to those described and shown in mice (14).

Other Tumors. In a few cases additional types of neoplasms were observed in the treated groups, (Table 2). Since they occurred rarely their appearance could not be attributed to the treatment.

DISCUSSION

These results clearly demonstrate that, in randomly bred Swiss mice, lifelong administration of 2% SADH in drinking water, from 6 weeks of age, induced tumors of blood vessels, lungs, and kidneys. The incidence of blood vessel tumors increased from 8 to 72% (p < 0.0001) in females and from 5 to 74% (p < 0.0001) in males, compared to untreated controls. In addition, 74% (p < 0.0001) of the treated females and 72% (p < 0.0001) of the treated males developed lung tumors, whereas among controls the corresponding incidence was 15% in females and 22% in males. Furthermore, 10% (p < 0.0066) of the treated males developed kidney tumors, while none were found in the controls. Statistical analysis was carried out by the use of Fisher's exact test for 2 x 2 tables (2). Histopathologically, the tumors were classified as angiomas and angiosarcomas of blood vessels, adenomas and adenocarcinomas of lungs and adenomas of kidneys. Since SADH hydrolyzes to a small amount of 1,1-dimethylhydrazine, one can not rule out the possibility that this latter chemical might be responsible to a limited extent for the tumorigenic effect. Needless to say, according to agricultural practice, Alar-85, the commercial formula of SADH, is also dissolved in water and kept for certain lengths of time.

The metabolism of 14C-labeled SADH has been studied in rats and cows. Based on measurement of radioactivity, the chemical or its metabolite were excreted almost entirely in the urine and feces. Small amounts of radioactivity were also present in various organs and in expired carbon dioxide (Ref. 13; F. H. Ryer and J. B. Sullivan, Hazelton Labora-
### Table 2

**Tumor distribution in SADH-treated and control Swiss mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Effective no.</th>
<th>Sex</th>
<th>Blood vessels</th>
<th>Lungs</th>
<th>Kidneys</th>
<th>Other tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Age at death</td>
<td>%</td>
<td>Age at death</td>
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<td></td>
<td></td>
<td></td>
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<td>No.</td>
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<td>No.</td>
<td>No.</td>
</tr>
</tbody>
</table>
| 1     | 2% SADH in drinking water daily for life | 50            | F   | 36            | 72    | 63       | 61         | 61 (32-96)  | 7 malignant lymphomas (44, 47, 53, 64, 70, 90)  
|       |                               |               |     | 50            | 37    | 74       | 36         | 5           | 2 hepatomas (49, 78)  
|       |                               |               |     |               |       | 37       | 74         | 5           | 1 malignant lymphoma (79)  
|       |                               |               |     |               |       | 36       | 72         | 10          | 1 adenoma of thyroid (56)  
|       |                               |               |     |               |       | 36       | 60         | 67 (41-83)  | 1 malignant histiocytoma (83)  
|       |                               |               |     |               |       |          |            |             | 2 fibrosarcomas, s.c. (101, 102)  
|       |                               |               |     |               |       |          |            |             | 2 cord mesenchyma tumors (107, 114)  
|       |                               |               |     |               |       |          |            |             | 1 adenocanthoma of ovary (71)  
|       |                               |               |     |               |       |          |            |             | 1 adenoma of ovary (110)  
|       |                               |               |     |               |       |          |            |             | 1 adenoma of thyroid (88)  
|       |                               |               |     |               |       |          |            |             | 1 adenocarcinoma of breast (127)  
|       |                               |               |     |               |       |          |            |             | 1 adenocarcinoma of duodenum (86)  
|       |                               |               |     |               |       |          |            |             | 1 carcinoma of glandular stomach (64)  
|       |                               |               |     |               |       |          |            |             | 8 malignant lymphomas (28, 62, 67, 78, 84, 91, 92, 112)  
|       |                               |               |     |               |       |          |            |             | 2 fibrosarcomas, s.c. (69, 92)  
|       |                               |               |     |               |       |          |            |             | 2 adenomas of thyroids (63, 92)  
|       |                               |               |     |               |       |          |            |             | 2 hepatomas (68, 94)  
|       |                               |               |     |               |       |          |            |             | 1 adenoma of parathyroid (78)  
| 2     | Untreated controls             | 100           | F   | 8             | 8     | 92       | 15         | 15          | 7 malignant lymphomas (44, 47, 53, 64, 70, 90)  
|       |                               |               |     | 100           | 5     | 5        | 22         | 22          | 2 hepatomas (49, 78)  
|       |                               |               |     |               |       | 75       | 70         | 70 (40-124) | 1 adenoma of ovary (110)  
|       |                               |               |     |               |       |          |            |             | 1 adenoma of thyroid (88)  
|       |                               |               |     |               |       |          |            |             | 1 adenocarcinoma of breast (127)  
|       |                               |               |     |               |       |          |            |             | 1 adenocarcinoma of duodenum (86)  
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|       |                               |               |     |               |       |          |            |             | 2 hepatomas (68, 94)  
|       |                               |               |     |               |       |          |            |             | 1 adenoma of parathyroid (78)  

* Average and range.  
† Numbers in parentheses, age at death in weeks.
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