Comparison of Methyl Salicylate and Benzene as Solvents for Methylcholanthrene*

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Among the many factors which may modify the action of carcinogens in the induction of tumors in mice, the agents in which the chemicals are carried must be considered. Because of its keratolytic properties and the facility of absorption through the intact skin, methyl salicylate was tested as a solvent for tumor-producing hydrocarbons. Methylcholanthrene was chosen as the carcinogen. A comparison was made of the carcinogenic activity of methylcholanthrene in methyl salicylate and in benzene. The criteria used for comparing the carcinogenic activities were the latent periods for papilloma formation and for the onset of malignant tumors as determined by progressive growth of nodules and microscopic post-mortem examination.

RESULTS

The average time from the first application of carcinogen to the appearance of papillomas was 160 days in the mice painted with methylcholanthrene in methyl salicylate (Table I). In those painted with methylcholanthrene in benzene this average time was 156 days. The average time between the beginning of treatment and the occurrence of malignant tumors was 214 days in the former group and 233 days in the latter. Of 28 mice living at the time of appearance of the first papilloma, 26 developed papillomas, and 22 eventually developed malignant tumors in the methylcholanthrene-methyl salicylate group. Of 25 mice painted with methylcholanthrene in benzene, 24 developed papillomas and 19 later developed malignancies. The average number of papillomas per individual was 2.8 in the first group and 2.3 in the second group. Malignant tumors arose at two sites in each group and at three loci in one mouse painted with methylcholanthrene in benzene. The tumors had coalesced in these mice before sections were taken. Both malignant tumors and papillomas arose in the general region painted. The former were usually infected and in many cases the mice were cachectic. The curves show the tumors occurring up to a given time calculated as a percentage of the number of mice alive at that time (Fig. 1).

### Table I: Results of Painting Mice with Methylcholanthrene Dissolved in Methyl Salicylate or Benzene

<table>
<thead>
<tr>
<th>Preparations</th>
<th>No. alive at 114 days</th>
<th>Average induction time in days</th>
<th>Average no. per mouse</th>
<th>Malignant tumors</th>
<th>Average induction time in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylcholanthrene in methyl salicylate</td>
<td>28</td>
<td>160</td>
<td>2.8</td>
<td>22</td>
<td>214</td>
</tr>
<tr>
<td>Methylcholanthrene in benzene</td>
<td>25</td>
<td>156</td>
<td>2.3</td>
<td>19</td>
<td>233</td>
</tr>
<tr>
<td>Methyl salicylate</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Benzene</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Time of development of first papilloma.

### Methods

Mice of 8 inbred strains were divided into 4 groups with nearly equal proportions of each strain in each group. Thirty-six were painted with methylcholanthrene in methyl salicylate, 25 with methylcholanthrene in benzene, 39 with methyl salicylate alone, and 25 with benzene alone. Five mgm. of methylcholanthrene were contained in 1 cc. of the solvent in each instance. The reagents were applied to the backs of the mice with a camel’s hair brush at biweekly intervals. Although less exact than application from a syringe, this procedure was less lethal. Treatment was continued for 400 days.

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1 Worthington (18) dropped an exact amount of methylcholanthrene in methyl salicylate on the dorsum of each of 50 mice of various inbred strains. Forty-nine of the mice died within the first 12 hours, apparently as a result of the toxicity of the methyl salicylate.

The time of appearance of the first papilloma and the time of beginning induration and formation of a subcutaneous mass were recorded. The subcutaneous tumors were excised, fixed in Bouin's fluid, and stained with hematoxylin and eosin in order to verify their nature.

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Five different types of tumors not including metastatic growths were found in mice receiving methylcholanthrene (Table II). One mammary adenocarcinoma was found in a CBAN female 139 days after the painting was begun. A papilloma occurred at 167 days in this animal. One extension to the pancreas and two metastases to the lung were observed. The tumors referred to as mixed in this paper are those in which two types of neoplastic tissue appear side by side in the microscopic sections. An interesting fact is that eosinophils, in which the nucleus was not lobed, sometimes appearing almost like that of a plasma cell, were found in all but three mice painted with methylcholanthrene in methyl salicylate and in all but one mouse painted with methylcholanthrene in benzene. In some cases the tissue eosinophilia was exceptionally pronounced, most of the cells appearing in the edematous corium, but some in the tumor tissue itself.

No tumor appeared in the mice painted with benzene or with methyl salicylate alone. These animals were treated for 400 days. At this time all mice painted with methylcholanthrene in one solvent or the other had either developed tumors or had died. Ten mice painted with methylcholanthrene in methyl salicylate died without developing papillomas or subcutaneous tumors. Eight of these died before the first papilloma appeared in the group. Only one mouse painted with methylcholanthrene in benzene failed to exhibit a papilloma, but this animal developed a malignant neoplasm.

Table II: Types of Tumors Resulting from Painting with Methylcholanthrene in Different Solvents

<table>
<thead>
<tr>
<th>Solution used</th>
<th>Epidermoid carcinoma</th>
<th>Spindle cell sarcoma</th>
<th>Mammary adenocarcinoma</th>
<th>Mixed tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylcholanthrene in methyl salicylate</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Methylcholanthrene in benzene</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1</td>
<td>0</td>
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<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>16</td>
</tr>
</tbody>
</table>

Discussion

In considering the effect of solvents for carcinogens (6, 13) discussion is confined to those papers in which authors have specifically tested the solvents under similar laboratory conditions.

Berенблюм and Kendal (4) found that mice showed more tumors when dibenzanthracene in colloidal solution was injected intraperitoneally than when injected in lard. On the other hand, Oberling and co-authors (10) found fewer tumors in mice injected with benzpyrene in the colloidal state than with the carcinogen in olive oil. Also Andervont and Lorenz (1) found that subcutaneous tumors appeared less rapidly when dibenzanthracene was injected dispersed in horse or dog serum than when injected in solution in lard. Tumors occurred more rapidly when dibenzanthracene dispersed in horse serum with added charcoal was injected subcutaneously in C3H mice than when a dispersion in horse serum alone was used (2).

Peacock (11) found that dibenzanthracene in lard evoked tumors in approximately 50 per cent of the fowls injected, whereas no tumors occurred when chicken fat was used as the solvent. In a continuation of the work, Peacock and Beck (11) found that fewer tumors occurred when benzpyrene was injected subcutaneously in mouse fat, ether, or in the powdered form than when the solvent was used. olive lipoids, or a 5 olive oil and paraffin mixture. Morton and Mider (9) substantiated this work in experiments on mice of the C57 strain, using a petroleum ether extract of mouse carcasses. However, Oberling et al. (10) found no difference in tumor incidence when rat fat, lard, and olive oil were used as solvents for benzpyrene injected into white rats. Also Shimkin and Ander-
greatly different in the two groups especially when one considers separately the types of tumor tissue found in the mixed tumors (Table II). For instance, there were 3 tumors containing spindle cell sarcoma in the methylcholanthrene-methyl salicylate-treated animals and 4 such tumors in animals treated with methylcholanthrene in benzene. The average number of papillomas in the two groups differs by only 0.5. Both groups contained mice in which malignancies arose from more than one site.

It is possible that a difference could be detected if smaller doses of methylcholanthrene had been applied, since the amount used may have been so overwhelming as to mask any effect of the solvent, methyl salicylate, as compared to benzene. It has been suggested in the literature (12) that lower levels of carcinogen would be required for such an effect to be detected.

**Summary**

Under the conditions of this experiment no difference was found in the tumor incidence, type, and time of appearance in mice painted biweekly with methylcholanthrene in methyl salicylate and methylcholanthrene in benzene.

**References**


18. Worthington, R. V. Personal communication.
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