Carcinogenicity of Multiple Chemicals Simultaneously Administered*

PAUL E. STEINER

(Department of Pathology, University of Chicago, Chicago 37, Ill.)

The possibility that several carcinogens may act concurrently in organs such as the lungs and skin has assumed greater importance as the recognized possible agents increase for these sites. A wide range of effects, from inhibition through no effects to summation or even synergism must be considered. The dosage, type of agents, portal of entry, duration of exposure, site and type of tissue, species and strain of animal, and many other factors enter into the problem.

This communication deals only with the carcinogenic activity of a few chemicals, polycyclic aromatic hydrocarbons, injected simultaneously in the same tissue and acting in the same locus, probably on the same cells. The agents were selected because they represent strong, moderate, weak, and noncarcinogenic chemicals of similar general type. Some other aspects of testing for summation and for inhibitory effects have previously been mentioned, together with additional references (8).

METHODS

All tests were performed on male and female mice of C57BL strain. They were fed laboratory chow and water and were kept, ten to fifteen in a cage, until a growing mass was detected, at which time they were isolated. They were weighed at monthly intervals; the findings obtained were not the result of differences in weight.

All tests were made by subcutaneous injection in the interscapular region. The solvent was always tricaprylin, and the total volume, 0.5 cc. To minimize loss from back-leakage, long needles were used of the smallest calibre (usually 26-gauge) consistent with the physical state of the material. Usually 40 or 50 mice were given injections in each test. The effective total in the tables refers to the number of mice alive in the experiment when the first tumor appeared. The experiments were terminated between the 22d and the 28th months by sacrificing all remaining animals. Only tumors at the injected site are included in the results, and all diagnoses were based on microscopic findings.

The quantities of the chemicals used were chosen, in the case of the strong carcinogens, because they were known to induce tumors in about 50 per cent of these mice and, in the other chemicals, because they were believed to be near the saturation dose levels. It was not possible to use all desirable combinations of dosage.

RESULTS

RESULTS FROM STANDARD DOSES

Strong plus medium carcinogens.—Three chemicals regarded as strong carcinogens were tested in combination with an agent of medium potency (1,2-benzanthracene), with the following results (Table 1).

Experiment 1: 1,2,5,6-Dibenzanthracene plus 1,2-benzanthracene showed no summation of activity. In fact, the combination was probably significantly less potent than was 1,2-benzanthracene tested alone, so that there may have been inhibition.

Experiment 2: The combination of 3-methylcholanthrene and 1,2-benzanthracene yielded significantly more tumors than did either alone (P > 0.01), indicating summation effects. The addition of effects was, however, imperfect (Experiments 2B plus 1C).

Experiment 3: When 3,4-benzpyrene and 1,2-benzanthracene were tested together, their individual potencies were not summated. The tumor yield was within the statistical range of either injected alone.

Strong plus weak carcinogen.—Experiment 4: The combination of 1,2,5,6-dibenzanthracene and chrysene showed no significant summation of effects. The yield of tumors was the same as for either agent alone, and the question can be raised whether there was not actual interference.

Strong plus inactive agents.—Experiment 5: Anthracene alone induced no tumors. When it was injected along with 1,2,5,6-dibenzanthracene, the
yield of tumors was not significantly different from that when the carcinogen was tested alone.

Experiment 6: Phenanthrene also induced no tumors. Tested in conjunction with 1,2,5,6-dibenzanthracene, there was no significant alteration of tumor response to the carcinogen.

RESULTS FROM DOUBLING THE DOSES

Experiment 7: In this experiment 1,2,5,6-dibenzanthracene and 1,2-benzanthracene were tested separately and together, in double the amounts of chemical used in Experiment 1 (Table 1). Ten mg. of 1,2-benzanthracene gave no higher percentage of induced tumors than 5.0 mg., and 0.04 mg. of 1,2,5,6-dibenzanthracene no more than 0.02 mg. of this compound. These results may be interpreted as indicating that the smaller doses were near "saturation." Injected together, however, there appears to have been a significant summation of carcinogenic effects.

COMMENTS

It is emphasized that the results here reported apply only to the agents, dosages, and other conditions of testing as described. They re-emphasize that carcinogenic activity is not something resident, fixed, and immutable in an agent but that it is a resultant of local conditions and cells. This is shown by an increase in tumor yield resulting from a reduction in dosage (Table 2). It is also suggested by the various responses obtained to a

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Chemical and quantity</th>
<th>Effective total no. of mice</th>
<th>No. of induced sarcomas (per cent)</th>
<th>Tumor induction time (days)</th>
<th>Av. induction time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>1,2,5,6-Dibenzanthracene (0.02 mg.) + 1,2-benzanthracene (5 mg.)</td>
<td>25</td>
<td>14</td>
<td>40</td>
<td>150</td>
</tr>
<tr>
<td>1B</td>
<td>1,2,5,6-Dibenzanthracene (0.02 mg.)</td>
<td>21</td>
<td>7</td>
<td>33</td>
<td>160</td>
</tr>
<tr>
<td>1C</td>
<td>1,2-Benzanthracene (5 mg.)</td>
<td>36</td>
<td>20</td>
<td>55</td>
<td>183</td>
</tr>
<tr>
<td>2A</td>
<td>20-Methylicholanthrene (0.02 mg.) + 1,2-benzanthracene (5 mg.)</td>
<td>30</td>
<td>25</td>
<td>83</td>
<td>150</td>
</tr>
<tr>
<td>2B</td>
<td>20-Methylicholanthrene (0.02 mg.)</td>
<td>27</td>
<td>14</td>
<td>52</td>
<td>114</td>
</tr>
<tr>
<td>3A</td>
<td>3,4-Benzpyrene (0.09 mg.) + 1,2-benzanthracene (5 mg.)</td>
<td>27</td>
<td>16</td>
<td>59</td>
<td>120</td>
</tr>
<tr>
<td>3B</td>
<td>3,4-Benzpyrene (0.09 mg.)</td>
<td>21</td>
<td>16</td>
<td>76</td>
<td>145</td>
</tr>
<tr>
<td>4A</td>
<td>1,2,5,6-Dibenzanthracene (0.02 mg.) + chrysene (5 mg.)</td>
<td>25</td>
<td>9</td>
<td>36</td>
<td>160</td>
</tr>
<tr>
<td>4B</td>
<td>Chrysene (5 mg.)</td>
<td>22</td>
<td>5</td>
<td>23</td>
<td>150</td>
</tr>
<tr>
<td>5A</td>
<td>1,2,5,6-Dibenzanthracene (0.02 mg.) + anthracene (5 mg.)</td>
<td>29</td>
<td>13</td>
<td>45</td>
<td>145</td>
</tr>
<tr>
<td>5B</td>
<td>Anthracene (5 mg.)</td>
<td>25</td>
<td>9</td>
<td>29</td>
<td>120</td>
</tr>
<tr>
<td>6A</td>
<td>1,2,5,6-Dibenzanthracene (0.02 mg.) + phenanthrene (5 mg.)</td>
<td>26</td>
<td>14</td>
<td>54</td>
<td>256</td>
</tr>
<tr>
<td>6B</td>
<td>Phenanthrene (5 mg.)</td>
<td>27</td>
<td>9</td>
<td>20</td>
<td>160</td>
</tr>
<tr>
<td>7A</td>
<td>1,2,5,6-Dibenzanthracene (0.04 mg.) + 1,2-benzanthracene (10 mg.)</td>
<td>18</td>
<td>6</td>
<td>33</td>
<td>150</td>
</tr>
<tr>
<td>7B</td>
<td>1,2,5,6-Dibenzanthracene (0.04 mg.)</td>
<td>16</td>
<td>5</td>
<td>31</td>
<td>207</td>
</tr>
</tbody>
</table>

* No. of mice surviving at 4 months.

carcinogen (1,2-benzanthracene, in this instance) tested at different times and with different lots of the chemical but in the same strain of mice and laboratory (7, 8).

Perhaps the most interesting finding was the progressive increase in tumor yield when the amount of 1,2-benzanthracene was reduced from 5.0 mg. to 0.05 mg. in the presence of a constant but small quantity (0.02 mg.) of 1,2,5,6-dibenzanthracene (Table 2). Because no similar increase but rather a decrease occurred with reduced doses of 1,2-benzanthracene tested alone, and because it exceeded the tumor yield for 1,2,5,6-dibenzanthracene alone, it is regarded as a summation phenomenon.

The present results confirm our previous findings that there is an absence of mutual effects between 1,2-benzanthracene and 1,2,5,6-dibenzanthracene when they are tested together in this
way (1). The other combinations had not previously been tested.

Most of the reports by others on additive or inhibitory carcinogenic effects of chemicals administered simultaneously to the same tissue have concerned either other agents or other anatomical sites. MacDonald et al. observed a synergistic action when some hepatic carcinogens were fed to rats (4), a situation that requires absorption and transportation of agent and also involves a different type of tissue. Following skin painting in mice, Lacassagne and co-workers observed an inhibition of the potency of some strong carcinogens by weak hydrocarbons (9). Riegel et al. (6) confirmed in part the results of Lacassagne, obtaining delay in methylcholanthrene-induced skin tumor formation by 1,2,5,6-dibenzofluorene but not by chrysene, fluorene, or 1,2,7,8-dibenzofluorene. The same workers (1) later studied the effects of nine dif-

ferent weak compounds on skin carcinogenesis by 9,10-dimethyl-1,2-benzanthracene and found that 1,2-benzanthracene prolonged the mean latent period for skin tumor formation by that compound and that anthracene did so slightly. When they tested strong carcinogens under similar conditions, these workers found that 1,2,5,6-dibenzanthracene, benzpyrene, and methylcholanthrene all delayed tumor appearance on the skin by 9,10-dimethyl-1,2-benzanthracene, although they had no effect on tumor yield (2).

Müller et al. attempted to formulate a chemical theory for the results of the investigations cited above and concluded that they might be explained on the basis of either intercrystallization or a simple eutectic (5). From a study of our results and those of others, it appears that general axioms cannot be recognized until more information is available.

SUMMARY

Aromatic polycyclic hydrocarbons in various combinations were tested for carcinogenic activity in the subcutaneous tissues of C57BL mice. Three strong carcinogens tested with a medium-strength chemical (1,2-benzanthracene) caused, variously, summation effects (with 20-methylcholanthrene) or no effect (1,2,5,6-dibenzanthracene; 3,4-benzpyrene). A strong carcinogen (1,2,5,6-dibenzanthracene) did not summate effects with a weak agent (chrysene). Chemically related but non-carcinogenic compounds (anthracene, phenanthrene) added to a strong carcinogen (1,2,5,6-dibenzanthracene) did not affect the tumor yield; they were not co-carcinogenic. Doubling the amount of each of two strong carcinogens increased the tumor yield over either alone. As the dose of a medium strength carcinogen (1,2-benzanthracene) was reduced, the tumor yield decreased. However, when these amounts of the chemical were tested with a constant quantity of a strong carcinogen (1,2,5,6-dibenzanthracene), no similar decrease followed but rather a summation of carcinogenic effects at the lower doses. Chrysene and 1,2-benzanthracene were again found to be carcinogenic, and anthracene and phenanthrene to be inactive. With the agents and dosages here used, no striking effects were observed on carcinogenic activity except when the dosage of one agent was greatly reduced (Experiment 8); here a summation effect was obtained.

REFERENCES


2. ———. Inhibition of Skin Carcinogenesis in Mice by Mixtures of Strong Carcinogens. Ibid., 12:470–71, 1952.


Carcinogenicity of Multiple Chemicals Simultaneously Administered

Paul E. Steiner


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/15/9/632

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.