The Lack of Effect of Urethan (Ethyl Carbamate) on Sarcoma Induction by the Subcutaneous Injection of 3,4-Benzpyrene*

I. BERENBLUM AND N. TRAININ

(Department of Experimental Biology, Isaac Wolfson Building, The Weizmann Institute of Science, Rehovoth, Israel)

SUMMARY

The incidence and induction time for sarcomas at the site of injection of 3,4-benzpyrene, in C57BL/6/Jax and C3H/Jax mice, were found to be independent of the effect of urethan, administered systemically, either prior or subsequent to the injection of the hydrocarbon.

Conversely, the induction of lung adenomas and of leukemia in C57BL/6/Jax and C3H/Jax mice that received multiple injections of urethan appeared to be independent of the effect of prior subcutaneous injection of benzpyrene.

In addition to its pronounced carcinogenic action on the lung (20, 26), urethan (ethyl carbamate) is also now known to have some carcinogenic action on certain other tissues. When fed to mice by stomach tube it induces squamous papillomas of the forestomach (3, 17); administered repeatedly by skin painting or intraperitoneal injection, it causes a moderate rise in the incidence of mammary tumors in both high- and low-mammary tumor strains and also mesenchymal tumors of the intrascapular fat pads and cystadenomas of the harderian gland (29, 31). Similar effects are obtained when the urethan is administered in the drinking water (30). A slight rise in the incidence of hepatomas, in strains of mice having a spontaneous incidence of this tumor, has been observed when urethan is administered to adults (18), and more strikingly so when it is administered to newborn mice (17, 19). The “blood cysts,” so often observed in livers of urethan-treated mice, have been described by some as non-neoplastic lesions (1, 8, 20) and by others as hemangiomas (25) or hemangioendotheliomas (6, 13). It has since been shown that typical “blood cysts” may yield transplantable hemangiomatous tumors (34). Urethan also possesses mild leukemogenic activity when administered to adult mice (30, 32), and very much more pronounced activity when the treatment is begun soon after birth (7, 10, 17, 19, 21).

In the rat, urethan induced hepatomas (13) as well as lung tumors (14, 24), whereas in the hamster it may produce skin melanomas (22, 33), tumors of the forestomach, adenomatous polyps of the cecum, hemangiosarcomas, and hepatomas (33). Some species are thought to be unresponsive to urethan carcinogenesis (12, 26, 28), although the numbers of tests performed in species other than the mouse and rat are insufficient to warrant such a definitive negative conclusion.

Urethan can also act as an “incomplete” carcinogen for certain tissues in mice—i.e., requiring the participation of certain additional forms of treatment for the carcinogenic effect to become manifested. It is a potent initiator for skin carcinogenesis when applied topically (1, 11, 25) or administered systemically (2), with croton oil serving as promoter. It also potentiates the leukemogenic action of total-body radiation when the two are administered concurrently (4, 5, 16), or when urethan treatment is begun 2 weeks after completion of the radiation treatment, though not when the sequence is reversed (4, 5). Urethan also potentiates the leukemogenic action of estrogens and of polycyclic aromatic hydrocarbons (16).

The broader aspect of a possible synergistic action of urethan and other carcinogens on different tissues of the body has so far not been investigated, except for the one attempt to demonstrate cocarcinogenic activity of urethan in relation to azo-dye liver carcinogenesis in rats (15). The present experiment was designed to examine this possibility with respect to sarcoma induction in mice by...
the subcutaneous injection of 3,4-benzpyrene, when preceded or followed by intraperitoneal injections of urethan.

MATERIALS AND METHODS

Two strains of mice, C57BL/6/Jax and C3H/Jax, each bred for several generations in this laboratory by brother-sister matings, were used for this experiment. They were chosen on the basis of earlier reports (9) that the former strain is less responsive, and the latter strain somewhat moreresponsive, than other strains tested for sarcoma induction by local injection of carcinogenic hydrocarbons. Males only were used, to avoid the complication of spontaneous mammary tumor development (in the case of the C3H mice). The animals were 8–10 weeks old at the beginning of

One group of each strain received a single injection of 0.3 ml. (30 mg. of urethan) 1 week before the benzpyrene injection; a second group of each strain received 0.2 ml., 10 times, at weekly intervals (as a total of 200 mg. of urethan), the first injection being given 1 week after the benzpyrene injection. The rationale of this procedure was that a single dose (as large as the animal could conveniently tolerate) would be sufficient to test for initiating action, whereas repeated doses, over a period of many weeks, would be required for effective promoting action (2). The control groups received a single injection of benzpyrene without any other treatment.

The animals were examined fortnightly for evidence of a tumor at the site of benzpyrene injection. When a palpable swelling appeared, the

TABLE 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. at start of experiment</th>
<th>Survivors at 50th week</th>
<th>Survivors at 60th week</th>
<th>Primary treatment</th>
<th>Secondary treatment</th>
<th>Local sarcomas/effective total*</th>
<th>Per cent</th>
<th>Latent period, mean (range) (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>33</td>
<td>25</td>
<td>22</td>
<td>Benzyrene</td>
<td>Urethan</td>
<td>8/33</td>
<td>24</td>
<td>26 (14–32)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>39</td>
<td>37</td>
<td>Benzyrene</td>
<td>Urethan</td>
<td>19/60†</td>
<td>32</td>
<td>33 (17–54)</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>19</td>
<td>15</td>
<td>Benzyrene</td>
<td>Urethan</td>
<td>10/26</td>
<td>38</td>
<td>30 (22–62)</td>
</tr>
<tr>
<td>C3H/Jax</td>
<td>33</td>
<td>21</td>
<td>16</td>
<td>Benzyrene</td>
<td>Urethan</td>
<td>18/33</td>
<td>55</td>
<td>22 (20–64)</td>
</tr>
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<td></td>
<td>55</td>
<td>36</td>
<td>21</td>
<td>Benzyrene</td>
<td>Urethan</td>
<td>30/55</td>
<td>55</td>
<td>29 (14–50)</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>21</td>
<td>17</td>
<td>Benzyrene</td>
<td>Urethan</td>
<td>15/29</td>
<td>52</td>
<td>29 (30–40)</td>
</tr>
</tbody>
</table>

* Effective total = number of animals alive at the time of appearance of the first tumor in the group.
† One sarcoma, which appeared at a distance from the site of injection of the benzpyrene, is not included.

The carcinogen 3,4-benzpyrene (Edcan Laboratories) was made up as a 0.07 per cent solution in tricaprylin (Calif. Corp. for Biochemical Research). A single dose of 0.1 ml. (0.07 mg. benzpyrene) was injected subcutaneously in the right axillary region in each animal. The dose chosen was based on earlier studies (18) which reported incidences of sarcoma, at the site of injection of, somewhat less than 50 per cent in C57BL, and slightly more than 50 per cent in C3H mice, at this dose level.

The urethan preparation (British Drug Houses, Ltd.) was made up as a 10 per cent solution in distilled water and administered by intraperitoneal injection. The dose per injection and number of injections differed according to whether the action of the urethan was to serve as initiator or promoter.

as a basis for comparison, the objective was to reach a median tumor response in the benzpyrene controls. The results (Table 1) show that this was approximated, with the induction of sarcomas in 24 per cent in the C57BL series and 55 per cent in the C3H series.

No evidence of augmentation or inhibition could, however, be detected in the experimental groups treated with urethan either before or after the subcutaneous injection of benzpyrene. In the C3H series, the figures for percentage of tumor incidence of the three groups were remarkably close; in the C57BL series, the actual differences were
small and not statistically significant when analyzed by the χ² test. The average latent periods also appeared closely similar in all the groups.

Lung adenomas were present in the majority of mice receiving multiple injections of urethan, but only occasionally in those that received a single injection.

In addition to localized sarcomas and lung adenomas, eight cases of leukemia were observed. Of these, four (out of 60 mice—i.e., 7 per cent) were in C57BL and three (out of 55—i.e., 5 per cent) were in CSH mice receiving benzpyrene followed by multiple injections of urethan, whereas one (out of 26 mice—i.e., 4 per cent) was in a C57BL mouse that received one injection of urethan followed by benzpyrene. Five of these eight leukemias were thymomas with or without generalized leukemia, one was a nonthymic generalized lymphatic leukemia, one a mesenteric lymphoma, and one was characterized by an enormous enlargement of the liver, with pronounced lymphatic infiltration but with little evidence of neoplastic changes elsewhere. (For comparison, the incidence of thymoma with or without generalized lymphatic leukemia in our untreated stock C57BL/6 mice was 0.7 per cent; those treated repeatedly with urethan alone, as controls for other experiments, developed an incidence of about 7 per cent.)

**DISCUSSION**

The purpose of the experiment was to determine whether the induction of sarcomas by the subcutaneous injection of 3,4-benzpyrene could be augmented by urethan, administered systemically, with the urethan acting either as an initiator, comparable to its action on the skin (1, 2, 24), or as a promoter, comparable to its effect in relation to leukemogenesis (4, 5). The results indicate that a summation or synergism, in terms of a two-stage process, did not occur.

Such results might be interpreted either as evidence of a failure of urethan to influence sarcoma induction, or alternatively, as a reflection of inadequate conditions operating in the particular experimental set-up. The fact (a) that the tumor incidence in the controls came within the median range (24 per cent for C57BL mice and 55 per cent for CSH mice) as planned, (b) that the cumulative dose of urethan used, at least in the test for promoting action, was high (200 mg.), and (c) that the treatment was continued, in this case, for a long time (10 weeks), leads one to assume that the negative result is a sign of failure on the part of urethan to influence sarcoma induction. A two-stage mechanism for sarcoma induction was thus not demonstrable under the experimental conditions employed.

The results of the two control groups confirm the earlier findings (9) that CSH mice are more responsive than C57BL mice to the induction of sarcomas with low doses of carcinogenic hydrocarbons, the difference in responsiveness being, however, small.

The development of lung adenomas and of leukemia, in some of the groups, can be completely accounted for by the action of urethan alone.

**REFERENCES**

15. ———. The Response of Rats to the Simultaneous Application of Two Different Carcinogenic Agents. Ibid., pp. 113-16.


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I. Berenblum and N. Trainin


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