Orally Administered 2-Acetylaminofluorene as an Initiator and as a Promoter in Epidermal Carcinogenesis in the Mouse*

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When certain carcinogenic hydrocarbons, one of which is 9,10-dimethyl-1,2-benzanthracene, are applied to mouse skin, they are able to prepare it so that subsequent applications of the noncarcinogenic substance croton oil become able to increase the number of tumors appearing in the prepared skin (5). Even a single application of one of these carcinogens, a dose too small to produce more than an occasional tumor, has been found adequate to prepare mouse skin so that subsequent applications of croton oil can elicit many skin tumors (4, 14). Berenblum and Shubik (4) suggested that such skin tumors were produced in two stages. In the first stage, called "initiation" (10), the carcinogen is thought to change some of the cells in the epidermis in such a way that in the second stage, called "promotion" (10), the noncarcinogenic croton oil is able to make these changed cells into tumors. Beta radiation (19) and urethan (17) are also able to produce the initial change in mouse skin, preparing it so that subsequent applications of croton oil become able to elicit skin tumors. Iodoacetic acid, chloracetophenone, and certain low-boiling fractions of petroleum (12, 20) have been reported to be able to "promote," eliciting tumors in mouse skin initiated by an application of 9,10-dimethyl-1,2-benzanthracene.

This paper reports experiments on the initiating and promoting power of 2-acetylaminofluorene in mouse skin. To test for initiating power, 2-acetylaminofluorene was fed for a time, and croton oil was applied to the skin subsequently. To test for promoting power, the skin was prepared by an application of 9,10-dimethyl-1,2-benzanthracene, and 2-acetylaminofluorene was fed subsequently. It should be noted that, though the croton oil and 9,10-dimethyl-1,2-benzanthracene were painted onto the skin, the 2-acetylaminofluorene was given by mouth and never applied directly to the skin. Some of the experiments have been briefly reported previously (16).

MATERIALS AND METHODS

Virgin, adult, male and female mice of three strains were used. The Oxford Swiss mice were of a strain originally obtained from the Medical Research Council's Laboratory at Mill Hill, London, and bred in the Sir William Dunn School of Pathology of the University of Oxford. The Bar Harbor Swiss mice and the CAF1 hybrids were bought from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine. The Oxford Swiss mice were kept in metal cages, the Bar Harbor Swiss and the CAF1 mice in plastic cages. In no case were the animals kept in a bright light. The Oxford Swiss mice were fed rat cake supplied by the North Eastern Agricultural Co-operative Society of Aberdeen, Scotland, the Bar Harbor Swiss and the CAF1 mice, Rockland Mouse Diet. Both food and water were given ad libitum. The experiments with the Oxford Swiss mice were performed at the Oxford University Research Centre of the British Empire Cancer Campaign, the Sir William Dunn School of Pathology, Oxford, and those with the Bar Harbor Swiss and the CAF1 mice in the laboratories of the Division of Oncology of the Chicago Medical School.

The 2-acetylaminofluorene was administered in the food, being added to the rat cake or mouse diet so as to make a final concentration of 0.024 per cent. The 2-acetylaminofluorene fed the Oxford Swiss mice was synthesized from fluorescein (British Drug Houses, Technical) according to the method of Blatt (6); that fed to the Bar Harbor Swiss and the CAF1 mice was obtained from the Eastman Kodak Company. While the Oxford Swiss mice were receiving 2-acetylaminofluorene, 50 mg. ascorbic acid, 1 mg. thiamine, 2 mg. riboflavin, 20 mg. nicotinamide, 2 mg. pyridoxine, and 3 mg. calcium pantothenate were added to each 20 cc. of the drinking water. This supplement was not given to the Bar Harbor Swiss or the CAF1 mice.

The croton oil was applied as a 5 per cent solution of oleum crotonis B. P. in mineral oil. The mineral oil used for the experiments with Oxford Swiss mice was liquid paraffin B. P. (Harrington), that used for the experiments with Bar Harbor Swiss or CAF1 mice, light mineral oil (Superl 34, Standard Oil of Indiana). Except as otherwise stated, the croton oil was applied with a paint brush to the whole back from ears to tail, the skin being kept free of hair with electric clippers. The croton oil was applied twice a week. 9,10-Dimethyl-1,2-benzanthracene (Light) was used as a 1.5 per cent solution in liquid paraffin B. P. (Harrington) and was also spread with a paint brush over the whole of the back from the ears to tail, the hair having been removed with electric clippers. It was applied once only.

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RESULTS

ACETYLAMINOFLUORENE AS AN INITIATOR

Experiment A.—An experimental group of 25 female Oxford Swiss mice was fed 2-acetylaminofluorene for 4 weeks. Applications of croton oil were then begun. Both the croton oil and the diet containing 2-acetylaminofluorene were continued for another 17 weeks. The 2-acetylaminofluorene was then withdrawn, and the croton oil continued alone until the mice died. A control group of 25 female Oxford Swiss mice was fed 2-acetylaminofluorene for 21 weeks but received no croton oil. A second control group of 25 male Oxford Swiss mice was given no 2-acetylaminofluorene but was painted with croton oil.

Skin tumors appeared in the experimental group both fed 2-acetylaminofluorene and painted with croton oil but no croton oil, and 22 of the control group given croton oil but no 2-acetylaminofluorene being alive 40 weeks after the start of the experiment. Nevertheless, no skin tumors were produced in any group.

It was thought that this failure to produce skin tumors might have been due to the use of a relatively resistant strain of mice. The experiment was therefore repeated on Bar Harbor Swiss mice, a strain known to be very sensitive to the initiating action of 9,10-dimethyl-1,2-benzanthracene.

Experiment C.—In the experimental group, 30 female Bar Harbor Swiss mice were fed 2-acetylaminofluorene for 50 days. After an interval of 7 days, applications of croton oil were begun and were continued for 41 weeks. A control group of 25 female Bar Harbor Swiss mice was fed 2-acetylaminofluorene for 50 days but received no further treatment. A second control group of 80 female Bar Harbor Swiss mice received no 2-acetylaminofluorene but was painted with croton oil for 41 weeks.

The findings are given in Table 2. Skin tumors appeared in the mice fed 2-acetylaminofluorene and then painted with croton oil but not in those only fed 2-acetylaminofluorene or only painted with croton oil. Ten skin tumors were present on five mice bearing skin tumors that died during the experiment, and three regressed, one of the regressions being on a mouse that bore no other tumor. The average latent period, as measured from the first application of croton oil, was 42 weeks. The skin tumors were all papillomas. Three subsequently became carcinomas.

Two other experiments with 2-acetylaminofluorene and croton oil failed to produce skin tumors. It should be noted that in both the croton oil was applied only to a small area in the interscapular region instead of to the whole back.

Experiment D.—Female Oxford Swiss mice were...
fed 2-acetylaminofluorene for 4 months. In ten of
the survivors, croton oil was applied to a small
area in the interscapular region. Croton oil and 2-
acetylaminofluorene were both continued for an-
other 8 months. No skin tumors were produced.

**Experiment E.**—One of the rare reports of skin
tumors (other than tumors of the ductus acusticus
externus in rats) occurring in animals fed 2-acetyl-
aminofluorene is that of Bielschowsky (5), who
saw three carcinomas of the skin in rats which
were also receiving p-aminobenzoic acid. Because
of this report, 50 female Oxford Swiss mice were
fed a diet which in addition to 2-acetylamino-
fluorene contained p-aminobenzoic acid (British
Drug Houses Laboratory Reagent) in such quan-
tity that each mouse received about 50 mg/day.
After 4 months on this diet, applications of croton
oil to a small area in the interscapular region were
begun in 25 of the mice. Both croton oil and the
diet were continued for another 8 months. No skin
tumors were seen.

**Acetylaminofluorene as a Promoter**

**Experiment F.**—In the experimental group, 25
female Oxford Swiss mice were painted once with
9,10-dimethyl-1,2-benzanthracene. One week later
2-acetylaminofluorene was started and was fed for
41 weeks. A control group of 49 female Oxford
Swiss mice was painted once with 9,10-dimethyl-
1,2-benzanthracene but received no 2-acetyl-
aminofluorene. A second control group of 60 fe-
male Oxford Swiss mice received no 9,10-di-

**TABLE 2**

**Acetylaminofluorene as an Initiator (Experiment C)**

<table>
<thead>
<tr>
<th>Time (in weeks)</th>
<th>Survivors bearing skin tumors</th>
<th>Skin tumors present on survivors</th>
<th>Total no. mice with skin tumors</th>
<th>Total skin tumors produced</th>
<th>Survivors produced</th>
<th>Skin tumors produced</th>
<th>Survivors produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>20</td>
<td>8 (31 per cent)</td>
<td>11</td>
<td>11</td>
<td>24</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>32</td>
<td>20</td>
<td>6 (30 per cent)</td>
<td>9</td>
<td>12</td>
<td>24</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>48</td>
<td>17</td>
<td>7 (41 per cent)</td>
<td>22</td>
<td>24</td>
<td>24</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>64</td>
<td>10</td>
<td>7 (70 per cent)</td>
<td>46</td>
<td>59</td>
<td>11</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

* Time is measured from the first application of croton oil in the first and third groups, and from the equivalent time 7 days after the withdrawal of 2-acetylaminofluorene in the second group.

† 2-Acetylaminofluorene fed for 50 days, then a 7-day interval, then applications of croton oil twice a week for 41 weeks.

‡ 2-Acetylaminofluorene fed for 50 days, but no other treatment.

§ Croton oil applied twice a week for 41 weeks, but no other treatment.

**TABLE 3**

**Acetylaminofluorene as a Promoter (Experiment F)**

<table>
<thead>
<tr>
<th>Time (in weeks)</th>
<th>Survivors bearing skin tumors</th>
<th>Skin tumors present on survivors</th>
<th>Total no. skin tumors</th>
<th>Total skin tumors produced</th>
<th>Survivors produced</th>
<th>Skin tumors produced</th>
<th>Survivors produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>17</td>
<td>1 (6 per cent)</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>56</td>
<td>17</td>
<td>3 (18 per cent)</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>42</td>
<td>16</td>
<td>5 (30 per cent)</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>64</td>
<td>15</td>
<td>9 (50 per cent)</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

* Time is measured from the start of the 2-acetylaminofluorene in the first and third groups, and from the equivalent time 7 days after the application of 9,10-dimethyl-1,2-benzanthracene in the second group.

† One application of 9,10-dimethyl-1,2-benzanthracene, then a 7-day interval, then 2-acetylaminofluorene fed for 41 weeks.

‡ One application of 9,10-dimethyl-1,2-benzanthracene, but no other treatment.

§ 2-Acetylaminofluorene fed for 41 weeks, but no other treatment.

Acetylaminofluorene was used as a promoter, and the findings are given in Table 3. Skin tumors appeared in both the groups painted with 9,10-dimethyl-1,2-benzanthracene, though not in the group given only 2-acetylaminofluorene. That a single application of 9,10-dimethyl-1,2-benzanthracene should produce a few skin tumors in Swiss mice is not surprising (7, 8, 13). What seems important is that more skin tumors were produced when the application of 9,10-dimethyl-1,2-benzan-
thracene was followed by feeding 2-acetylaminofluorene, there being only five skin tumors in the 28 survivors given only 9,10-dimethyl-1,2-benzanthracene, but thirteen in nine of the fifteen survivors painted with 9,10-dimethyl-1,2-benzanthracene and then fed 2-acetylaminofluorene. No skin tumor regressed, and no mouse bearing a skin tumor died during the experiment. The average latent period of the skin tumors in the experimental group was 43 weeks as measured from the start of the 2-acetylaminofluorene. The first skin tumor to appear in the experimental group was a carcinoma at the time of its first appearance, but the others were all papillomas when they first appeared. Subsequently, three became carcinomas. No tumor in the control group painted with 9,10-dimethyl-1,2-benzanthracene but given no 2-acetylaminofluorene became a carcinoma.

**DISCUSSION**

The skin tumors were of the kinds usually seen when tumors are induced in mouse skin by repeated applications of a carcinogenic hydrocarbon or by an application of such a hydrocarbon followed by repeated applications of croton oil (18). In the mice fed 2-acetylaminofluorene, many tumors appeared in the internal organs. These were usually of the liver, hepatomas and cholangiomas, but carcinomas or papillomas were also seen in the breast, ovary, bladder, and pancreas. One osteogenic sarcoma arose in the sacrum. No abnormality was detected in the skin of twelve Bar Harbor Swiss mice fed 2-acetylaminofluorene for 94 weeks but given no other treatment—in particular, there was no hyperplasia, the epidermis remaining one to two cells thick.

**SUMMARY**

1. Orally administered 2-acetylaminofluorene was able to prepare mouse skin so that subsequent topical applications of croton oil became able to elicit skin tumors.

2. Orally administered 2-acetylaminofluorene was able to increase the number of tumors appear-
ing in mouse skin prepared by a previous application of 9,10-dimethyl-1,2-benzanthracene.

3. Alone, orally administered 2-acetylamino-fluorene was unable to produce tumors of mouse skin.

4. The significance of these findings is discussed.

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