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

A. C. Ritchie† and Umberto Saffiotti

(Division of Oncology, The Chicago Medical School, 2755 W. 15th Street, Chicago 8, Ill.)

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 (3). 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-acetylamino fluorene in mouse skin. To test for initiating power, 2-acetylamino fluorene 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-acetylamino fluorene 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-acetylamino fluorene 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 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-acetylamino fluorene 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-acetylamino fluorene fed the Oxford Swiss mice was synthesized from fluorene (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-acetylamino fluorene, 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 oz. 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 (Super 84, 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.

* Part of this investigation was aided by a research grant from the National Cancer Institute, the National Institutes of Health, Bethesda, Md.
† Present address: Department of Pathology, Pathological Institute, McGill University, Montreal, Canada.

Received for publication July 30, 1954.

84
RESULTS

ACETYLAMINOFLUORENE AS AN INITIATOR

Experiment A.—An experimental group of 25 female Oxford Swiss mice was fed 2-acetylamino-
fluorene 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-acetylamino-
fluorene 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-acetylamino-
fluorene 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 fluorine but no croton oil, and 22 of the control group given croton oil but no 2-acetylamino-
fluorene 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 rela-
tively 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-acetyl-
aminofluorene 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-acetyl-

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACETYLAMINOFLUORENE AS AN INITIATOR (EXPERIMENT A)</td>
</tr>
<tr>
<td><strong>Time in weeks</strong></td>
</tr>
<tr>
<td>25</td>
</tr>
<tr>
<td>35</td>
</tr>
<tr>
<td>45</td>
</tr>
<tr>
<td>55</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 4 weeks after starting 2-acetylamino-

fluorene in the second group.

\[1\] 2-Acetylaminofluorene fed for 4 weeks, then 2-acetylaminofluorene fed and croton oil applied twice a week for 17 weeks, then croton oil only until the end of the experiment.

\[2\] Croton oil applied twice a week throughout, but no other treatment.

\[3\] Croton oil applied twice a week throughout, but no other treatment.

croton oil but not in either of the control groups (Table 1). Seven skin tumors were on the two mice bearing skin tumors that died during the experiment, and five regressed, two of the regressions being on one of the mice that died. The average latent period was 43 weeks, as measured from the first application of croton oil. The skin tumors were all papillomas. None became a carcinoma.

Experiment B.—To confirm that 2-acetylamino-
fluorene given by mouth could “initiate” in mouse skin, an experimental group of 22 female and seven male CAF, hybrids was fed 2-acetylamino-
fluorene for 20 weeks. After an interval of 4 weeks, croton oil was started and was continued until the mice died. A control group of fourteen female and six male CAF, hybrids was fed 2-acetylamino-
fluorene for 20 weeks but after a 4-week interval was painted with mineral oil instead of croton oil. A second control group of eight female and eighteen male CAF, mice was given no 2-acetylamino-
fluorene but was painted with croton oil. The mice survived well—23 of the experimental group given 2-acetylamino-
aminofluorene for 50 days but received no further treatment. A second control group of 30 female Bar Harbor Swiss mice received no 2-acetylamino-
fluorene but was painted with croton oil for 41 weeks.

The findings are given in Table 2. Skin tumors appeared in the mice fed 2-acetylamino-
fluorene and then painted with croton oil but not in those only fed 2-acetylamino-
fluorene 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 when they first appeared. Three subsequently became carcinomas.

Two other experiments with 2-acetylamino-
fluorene and croton oil failed to produce skin tu-
mors. It should be noted that in both the croton oil was applied only to a small area in the inter-
scapular 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 another 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-acetylaminofluorene 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-acetylaminofluorene contained p-aminobenzoic acid (British Drug Houses Laboratory Reagent) in such quantity 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.

### Table 2

<table>
<thead>
<tr>
<th>TIME in WEEKS</th>
<th>Survivors</th>
<th>Skin tumors present on skin survivors</th>
<th>Total no. mice with skin tumors produced</th>
<th>Total skin tumors produced</th>
<th>Survivors produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>20</td>
<td>8 (51 per cent)</td>
<td>11</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>32</td>
<td>20</td>
<td>6 (50 per cent)</td>
<td>9</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>48</td>
<td>17</td>
<td>7 (41 per cent)</td>
<td>22</td>
<td>34</td>
<td>15</td>
</tr>
<tr>
<td>64</td>
<td>10</td>
<td>7 (70 per cent)</td>
<td>46</td>
<td>59</td>
<td>11</td>
</tr>
</tbody>
</table>

* Time is measured from the start of the 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 41 weeks, but no other treatment.

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

### Table 3

<table>
<thead>
<tr>
<th>TIME in WEEKS</th>
<th>Survivors</th>
<th>Skin tumors present on skin survivors</th>
<th>Total no. mice with skin tumors produced</th>
<th>Total skin tumors produced</th>
<th>Survivors produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>17</td>
<td>1 (6 per cent)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>36</td>
<td>17</td>
<td>3 (18 per cent)</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>42</td>
<td>16</td>
<td>5 (25 per cent)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>48</td>
<td>15</td>
<td>9 (50 per cent)</td>
<td>9</td>
<td>18</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, 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.

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-benzanthracene was started and was fed for 41 weeks.
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 48 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.

HISTOLOGY

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 84 weeks but given no other treatment—in particular, there was no hyperplasia, the epidermis remaining one to two cells thick.

DISCUSSION

The experiments show that 2-acetylaminofluorene given by mouth is able to initiate tumors in mouse skin. Skin tumors were produced when 2-acetylaminofluorene was followed by croton oil, but never with 2-acetylaminofluorene alone or croton oil alone. The result of the experiment to investigate the promoting power of orally administered 2-acetylaminofluorene in mouse skin is less clear, for skin tumors appeared not only in the group painted with 9,10-dimethyl-1,2-benzanthracene and then fed 2-acetylaminofluorene but also in the control group given only 9,10-dimethyl-1,2-benzanthracene. The difference between the incidence of skin tumors in the control group and in the experimental group is probably large enough to suggest that 2-acetylaminofluorene given by mouth has some promoting power in mouse skin. Be that as it may, the experiments widen the application of the two-stage theory of epidermal carcinogenesis in mouse skin in that yet another substance has been found able to take part in the production of skin tumors by a "two-stage" mechanism.

Since the 2-acetylaminofluorene was administered in the food and since some soiling of the skin with food is inevitable, it might be considered that the 2-acetylaminofluorene was actually administered not only by mouth but also by direct contact with the skin. However, the quantity of 2-acetylaminofluorene reaching the skin must have been so small, and its concentration so low, that this soiling is probably of little significance. Price (15) found that repeated applications of a solution of 2-acetylaminofluorene were unable to produce tumors in mouse skin, even when followed by applications of croton oil. Salaman and Roe (17) produced no skin tumors in mice when two or four applications of a 2 per cent or of a saturated solution of 2-acetylaminofluorene were followed by repeated applications of croton oil; and Graffi, Vlamynck, Hoffman, and Schultz (11) were unable to produce significantly more skin tumors in mice by applying 2-acetylaminofluorene and croton oil alternately than with croton oil alone. If such large doses of 2-acetylaminofluorene were unable to produce skin tumors when applied directly to the skin either with or without croton oil, it is unlikely that the small quantities of 2-acetylaminofluorene reaching the skin from the dust of the diet were of importance.

It is of interest that, although 2-acetylaminofluorene given by mouth can initiate and probably promote tumors in mouse skin, it is unable to produce them unaided. Not only were no skin tumors seen in the various control groups fed 2-acetylaminofluorene in these experiments, but none have been reported in other experiments in which the drug was given to mice (1, 2, 9, 11, 15, 17, 21-23). It must also be remembered that 2-acetylaminofluorene seems unable to exert its initiating action when applied directly to mouse skin (11, 15, 17), only becoming adequate when given by mouth under favorable circumstances. Thus, it is apparent that tests for the carcinogenic action of the drug on mouse skin would be misleading if confined to its topical application and if its effect in combination with other promoters and other initiators were ignored.

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-acetylaminofluorene was unable to produce tumors of mouse skin.

4. The significance of these findings is discussed.

ACKNOWLEDGMENTS

Our thanks are due to Messrs. H. W. Wheel, R. Feldman, and I. Melancon for their valuable technical assistance.

REFERENCES


13. Law, L. W. Multiple Skin Tumors in Mice Following a Single Painting with 9,10-Dimethyl-1,2-benzanthracene. Am. J. Path., 17:827—31, 1941.


23. ——. The Carcinogenic Activity of 2-Acetylaminofluorene. III. Manner of Administration, Age of Animals and Type of Diet. Ibid., pp. 450—52.
Orally Administered 2-Acetylaminofluorene as an Initiator and as a Promoter in Epidermal Carcinogenesis in the Mouse

A. C. Ritchie and Umberto Saffiotti


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/15/2/84

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.