SKIN TUMORS IN ACI/N RATS INDUCED BY 3-METHYLCHOLANTHRENE AND 4-DIMETHYLAMINOSTILBENE*1

(Plates LXI–LXIV)

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Synopsis

1) Skin tumor developed in ACI/N rats that received 3-methylcholanthrene by skin painting and that plus 4-dimethylaminostilbene in the diet.
2) Trichoepitheliomas were much more common in animals painted with 3-methylcholanthrene and basal cell carcinomas were much more common in the group with 3-methylcholanthrene painting and 4-dimethylaminostilbene.

INTRODUCTION

There are only a few published reports concerning the induction of skin carcinoma in rats by the skin-painting method. Howell3) described his results with 3-methylcholanthrene and 7,12-dimethylbenz[a]anthracene (DMBA) in 1962, Fare and Orr1) in 1965 used 3-methoxy-4-dimethylaminoazobenzene, and Fare2) in 1966 reported the response to azo dyes.

Through dietary administration of N, N'-2, 7-fluorenylenebisacetamide (2,7-FAA), skin tumors were induced in ACI/N rats by How and Snell.4) In 1962, Odashima5) reported development of multiple skin tumors in rats that received skin paintings of 3-methylcholanthrene followed by oral administration of 4-dimethylaminostilbene.

The purpose of this report is to describe skin tumor development in ACI/N rats that received (1) 3-methylcholanthrene by skin painting, and (2) 3-methylcholanthrene by skin painting, plus 4-dimethylaminostilbene in the diet.

MATERIALS AND METHODS

Animals Eighty-six ACI/N rats (44 males and 42 females) were used in this experiment. They were housed 3 to a cage, and given diet and water ad libitum.

3-Methylcholanthrene Painting A solution of 0.3% 3-methylcholanthrene in acetone was used. The animals were painted twice weekly on the dorsal skin between the scapulae. This was done by dripping 1 ml of the solution from a syringe on the shaved skin.

4-Dimethylaminostilbene Diet 4-Dimethylaminostilbene dissolved in olive oil was added to the standard laboratory powder meal in quantity sufficient to give a final content of 0.05% by weight.

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Groups and Treatment The animals were divided into four groups treated as follows:

Group 1 (11 males and 9 females): 3-Methylcholanthrene painting for 20 weeks.
Group 2 (7 males and 11 females): 3-Methylcholanthrene painting and 4-dimethylaminostilbene diet simultaneously for 20 weeks.
Group 3 (12 males and 8 females): 4-Dimethylaminostilbene diet alone for 20 weeks.
Group 4 (14 males and 14 females): Acetone painting alone for 20 weeks, as controls.

Histological Preparations All the tumors, together with surrounding skin, were removed and fixed in Formalin. Sections cut at 5 μ were stained with Hematoxylin and Eosin, Alcian Blue, periodic acid-Schiff (PAS) reaction, and Sudan III for fat.

RESULTS

The tumors found in each experimental group are tabulated according to anatomical location and sex of the host in Table I.

Table I. Number of Rats and Incidence of Tumors

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial no. of animals</th>
<th>Effective no. of animals</th>
<th>Average intake of DAS (mg/rat)</th>
<th>Skin</th>
<th>Earduct</th>
<th>Liver</th>
<th>Breast</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20 § 11 9 8</td>
<td>19 § 11 8 8</td>
<td>0</td>
<td>19 § 11 8 8</td>
<td>0 0 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>18 § 7 17 § 6</td>
<td>91 17 § 6</td>
<td>91</td>
<td>§ 11</td>
<td>0 3 § 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>20 § 12 17 § 11</td>
<td>90</td>
<td>0</td>
<td>8 § 7 6 § 4</td>
<td>2 § 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>28 § 14 28 § 14</td>
<td>57</td>
<td>0</td>
<td>0 1 2 0</td>
<td>0 0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

DAS = 4-Dimethylaminostilbene.

Skin Tumors In Group 1, 1 out of 20 rats died before the 10th week without tumor development. In the remaining 19, skin tumors appeared during the 30th to 40th week, with an average latent period of 32 weeks from first skin painting. In Group 2, 1 out of 18 rats died before the 12th week, without tumor. In the remaining 17, skin tumors appeared during the 28th to 40th week, with average latent period of 30 weeks. In Group 3, 3 out of 20 rats died before the 10th week without tumor. In the remaining 17, no skin tumors appeared in the painted area by the time the experiment was terminated. However, in 8 of 17 rats in this group, ear-duct tumors were found. In Group 4 no skin tumors were observed.

Among the animals painted with 3-methylcholanthrene, loss of hair was the only grossly observable effect on the skin until the tumors appeared.

Skin tumors were first detected as hard nodules, 1-2 mm in diameter, usually covered by hairless epidermis. Growth was relatively slow, but eventually central ulceration developed in many of the nodules, as illustrated in Photos 1, 2, and 3.

When skin tumors occurred they were usually multiple. The average number of nodules per rat was 5.9 for Group 1 and 7.0 for Group 2.
SKIN TUMORS IN ACI/N RATS

Histological examination of the skin tumors revealed a wide variety of types. These have been classified according to the system proposed by Howell for skin tumors induced in rats by 3-methylcholanthrene and DMBA. In the present experiment, the most common histological types were trichoepithelioma and basal cell carcinoma.

The trichoepitheliomas were often difficult to distinguish from basal cell carcinomas. In the more highly differentiated examples, squamous cell nests with central cystic spaces containing keratin were commonly found. Such areas had a close resemblance to hair follicles. In the least differentiated examples, basal cell proliferation was more extensive, squamous epithelial nests were fewer, and there was no tendency to form structures resembling hair follicles.

Tumors less than 5mm in diameter were usually found to fall into the basal cell type. The nucleus in this cell type was oval or spindle shaped, and sometimes the nucleolus was prominent. Mitotic figures were numerous. In some tumors, the cells were arranged in palisades, especially at the margins of tumor cell masses. In some of these tumors there was a slight tendency toward keratinization.

Keratoacanthomatous tumors were common in this experiment. This type was composed of irregular, papillary projections of squamous epithelium, frequently showing hyperkeratosis and parakeratosis.

Sebaceous tumors were composed of well differentiated sebaceous cells with typical foamy, fat-containing cytoplasm and large central nuclei.

Total 112 tumors were examined in Group 1, which received 3-methylcholanthrene painting only. These included, besides the tumors described above, squamous cell carcinomas and hair follicle cyst.

In Group 3, which received the 4-dimethylaminostilbene diet in addition to 3-methylcholanthrene painting, 119 tumors were examined. As compared with tumors in Group 1, a much greater proportion (44.3% vs. 27.3%) was classified as basal cell carcinoma. Other tumors found in Group 2 were trichoepitheliomas, keratoacanthomas, sebaceous cell tumor, squamous papilloma, and hair follicle cyst.

Other Tumors Besides skin tumors, 8 examples of ear-duct tumors, 9 of liver tumors, and 2 of mammary tumors were found. These were all in rats of Groups 2 and 3.

Histologically, the tumors in ear ducts were typical squamous cell carcinomas, and the hepatic tumors were liver cell carcinomas.

Table II. Histological Classification of Skin Tumors

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratoacanthoma</td>
<td>10.6%</td>
<td>13.1%</td>
</tr>
<tr>
<td>Trichoepithelioma</td>
<td>50.0</td>
<td>21.3</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
<td>27.3</td>
<td>44.3</td>
</tr>
<tr>
<td>Sebaceous cell tumor</td>
<td>7.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>3.0</td>
<td>16.4</td>
</tr>
<tr>
<td>Squamous papilloma</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>Cyst of hair follicles</td>
<td>1.5</td>
<td>1.6</td>
</tr>
</tbody>
</table>
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Discussion

This study confirms that the rat is capable of developing neoplasms in response to a carcinogenic hydrocarbon 3-methylcholanthrene applied to the skin. Howell's earlier study did not only demonstrate this fact, but also showed that the type of neoplastic response varied according to the chemical composition of the carcinogen applied to the skin. Thus, in his work trichoepithelioma was the most common tumor type after 3-methylcholanthrene painting, while keratoacanthoma was the most common type after DMBA painting.

In How and Snell's experiments, 2,7-FAA was administered orally, but the carcinogenic effect was manifested in the skin as well as in other organs, illustrating again the well-known fact that carcinogens can produce tumors remote from the site of application, if they are absorbed and distributed systemically.

In the present work, the most interesting result was that the simultaneous dietary administration of 4-dimethylaminostilbene seemed to exert a modifying influence on the effect of topically-administered 3-methylcholanthrene, so far as skin tumors were concerned. Although 4-dimethylaminostilbene induced no skin tumors by itself, when given along with topical 3-methylcholanthrene there was a shift in the most common histologic type of skin tumor produced. With 3-methylcholanthrene alone, trichoepitheliomas were most frequent (44.3%), while with combined 3-methylcholanthrene and 4-dimethylaminostilbene, basal cell carcinoma was the most frequently found type (27.3%). It is also noteworthy that after 3-methylcholanthrene plus 4-dimethylaminostilbene, squamous carcinomas were about 5 times more frequent than after topical 3-methylcholanthrene alone.

There are several possible ways to interpret these findings. Perhaps the simplest and most attractive is to attribute to 4-dimethylaminostilbene the effect of decreasing the degree of differentiation in the group of tumors derived from basal cells (basal cell carcinoma and trichoepithelioma), and perhaps of having an additive or synergistic effect on the response of squamous epithelium to 3-methylcholanthrene. The 4-dimethylaminostilbene did not have an over-all additive or synergistic effect, since the frequency of skin tumors in the doubly-treated animals was no higher than for those given 3-methylcholanthrene paintings, 5.9 vs. 7.0.

Since the numbers of animals used in this experiment were relatively small, it seems advisable to collect further data on more animals similarly treated before attempting too precise an interpretation of the results.

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References

2) Fare, G., ibid., 26, 2406 (1966).
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EXPLANATION OF PLATES LXI~LXIV

Photo 1. Appearance of skin tumors of rat sacrificed on the 35th week in Group 2.
Photo 2. Gross appearance of multiple skin tumors in Group 1, sacrificed on the 32nd week.
Photo 3. Appearance of ulcerated skin tumors in Group 1, sacrificed on the 32nd week.
Photo 4. Basal cell proliferation from the epidermis.
Photo 8. Trichoepithelioma, similar to Photo 7. H–E. ×100.
Photo 9. Sebaceous tumor. Tumor is composed of variegated mixture of undifferentiated cells, sebaceous cells, and connective tissue. H–E. ×100.

H–E: Hematoxylin and Eosin stain.