9,10-Dimethyl-1,2-Benzanthracene as a Highly Potent Carcinogen for the Rabbit's Skin*

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There are many types of investigation connected with the tumor problem for which a large laboratory animal such as the rabbit would be more useful than a small one such as the mouse. For problems connected with experimental carcinogenesis the limiting factor is, naturally, the availability of a suitable carcinogen.

Neither tar nor 3,4-benzpyrene can be considered as suitable for the rabbit: The objection to the former is that it is a complex mixture of varying composition, action, and toxicity; to the latter, that its action on the rabbit is very weak. As for the many other polycyclic hydrocarbons that are known to produce skin tumors in mice, few seem to have been tested on the skin of the rabbit (2). Hence it is not possible to say from previous studies whether the rabbit can or cannot be extensively used for the detailed investigation of carcinogenesis.

It has been established that for mouse skin the most potent of the known agents is 9,10-dimethyl-1,2-benzanthracene (1, 3). It seemed desirable, therefore, to test this compound on the rabbit's skin also, and to compare the results with those obtained with tar and benzpyrene.

EXPERIMENTAL

The animals used for these experiments were young adult white rabbits, bred in this laboratory from a common stock and thus constituting a fairly homogeneous, though not genetically pure, strain. Their diet varied according to seasonal supplies, and included hay, oats, bread, cabbage, clover, mangolds, and carrots.

All solutions employed were kept in tightly-corked, dark bottles. They were applied to the skin with a Pasteur pipette, the amount deposited (about 8 drops) being just sufficient to cover most of the inner surface of the ear. All applications were made at half-weekly intervals.

RESULTS

9,10-Dimethyl-1,2-benzanthracene (1 per cent in benzene) was applied for 26 weeks to the right ears of 5 rabbits. Within a few seconds of the first application the treated area of skin became hyperemic, and this was followed soon after by a localized edematous swelling. These changes, evident after every application and persisting to some extent during the intervening periods, began to assume relatively less prominence as the epithelium became thickened and covered with loosely adhering, glistening flakes of keratin. This flaky hyperkeratosis became progressively more pronounced with each application.

Warts began to arise at the site of application at an early date, the first tumors appearing after 5, 5, 8, 6, 7, and 9 weeks respectively in the 5 rabbits. Many more tumors subsequently developed in all cases, until the inner surface of each treated ear became filled with a crowded collection of excrescences of different shapes and sizes, and of varying degrees of cornification. Many of these became confluent, and in later stages acquired the character of massive horny outgrowths (Fig. 1). In 1 rabbit the growths remained benign till the end of the experiment (26 weeks), but unmistakable signs of malignancy became apparent in the other 4 after the 16th, 16th, 19th, and 21st weeks respectively.

Subsequent histological examination of the lesions confirmed the malignant nature of the growths, as evidenced by their anaplastic character, deep penetration down to the cartilage (Figs. 2, 3, and 4), and in 2 cases through the cartilage to the other side. One of these led to the development of a large tumor on the outer side of the ear, which finally ulcerated to the surface. Metastases were not found in any of the animals.

Tar (diluted with a little benzene for ease of application) was applied for 36 weeks to the left ears of 6 rabbits. These rabbits also received applications of 25 per cent turpentine in acetone for the same period to the right ears. No tumors appeared on the right ears, treated with turpentine, but on the left ears, treated with tar, warts began to arise after 8, 11, 12,
Fig. 1.—Rabbit 71. 9,10-Dimethyl-1,2-benzanthracene twice weekly to the right ear for 24 weeks. Note massive horny growth protruding from right ear, and evidence of its extension through the cartilage to outer side of ear.

Fig. 2.—Rabbit 71. Part of tumor shown in Fig. 1. Note highly keratinizing character of growth. Mag. × 25.

Fig. 3.—Rabbit 71. Same as Fig. 2; higher magnification. Note invasion of carcinomaous growth down to the cartilage. Mag. × 150.

Fig. 4.—Rabbit 72. 9,10-Dimethyl-1,2-benzanthracene twice weekly to right ear for 26 weeks. Another squamous cell carcinoma showing invasion down to the cartilage. Mag. × 150.
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12, 16, and 29 weeks respectively. Neither the hyperemic nor the edematous changes were as intense as in the case of 9,10-dimethyl-1,2-benzanthracene, and the hyperkeratoses, though well developed, was less flaky in character. Multiple warts developed in the ensuing weeks, but they were few in number, their rate of growth was slow, and malignancy developed in only 1 case, at about the 30th week of application.

3,4-Benzpyrene (0.5 per cent in acetone, benzene, or xylene). This section includes an assorted collection of experiments in which benzpyrene was applied as part of an investigation unconnected with the present work. However, they may conveniently serve as controls for the present work, since the animals used were of the same stock and kept under similar conditions of diet, housing, and so forth.

First series.—In 6 rabbits the left ears were treated for 17 weeks with benzpyrene dissolved in benzene, and then for 16 weeks with the same substance dissolved in acetone. In 4 of these rabbits the right ears were treated for 47 weeks with benzpyrene in acetone, while the other 2 received an intravenous injection of 10 ml. of a saline extract of Shope papilloma virus.

None of these animals developed any tumors.

Second series.—In 7 rabbits the right ears were treated with benzpyrene in xylene for periods ranging from 39 to 48 weeks. At the same time the left ears were treated as follows: in 2 rabbits, applications of benzpyrene in acetone for 14 weeks with no further treatment; in 3 rabbits, the same treatment for 39 weeks; in the remaining 2 rabbits, the same treatment for 4 weeks, followed by applications of 5 per cent croton oil in acetone for a further 10 weeks.

A small wart developed in one of the rabbits after 33 weeks, at the site of application of benzpyrene in acetone; a wart appeared also in another rabbit after 48 weeks, at the site of application of benzpyrene in xylene. No other tumors were observed.

Thus among the 13 rabbits (20 ears receiving benzpyrene for 33 to 48 weeks) tumors were observed in only 2 cases.

DISCUSSION

The present investigation is concerned primarily with the carcinogenic action of 9,10-dimethyl-1,2-benzanthracene on the rabbit's skin, and the results obtained with 3,4-benzpyrene and with tar are presented merely for comparison. It would be desirable, therefore, to check the results for tar and benzpyrene with those of other workers.

Owing to variations in composition, and therefore in potency, of the different samples of tar used by various workers, published results on its carcinogenic action are very varied (14), and it is obviously impossible to quote a standard potency for this agent. Most observers would agree, however, that warts often appear early in rabbits after tar painting, but that such warts do not readily become malignant as they do, for instance, in mice. Hence when warts begin to arise from about the eighth week of application, as obtained in the present tar experiment, the tar in question may be considered as of more than average potency.

With regard to 3,4-benzpyrene, published results of carcinogenicity tests on rabbits are also conflicting, despite the fact that this substance is a single chemical compound. Thus benign and even malignant tumors have been produced by some workers (4, 5, 11, 12), yet not by others (6, 7, 9, 10). It is probable, however, that the failures were due to inadequate duration of the painting. The most reliable data seem to be those of Schürch (11), who painted 20 rabbits with benzpyrene until all the survivors had acquired tumors. He obtained benign growths in 6 to 14 months, with an average period of 11 months, and malignant tumors in 13 to 45 months, with an average period of 27 months. In the present experiments with benzpyrene, most of the animals were treated for less than 11 months, and the yield of tumors (2 among 20 ears of 13 rabbits) seems somewhat low by comparison, though probably not significantly so.

The results of the present investigation are of interest also from the point of view of species response. For purposes of comparison, the available data concerning the response of the skin of the mouse and rabbit respectively to the 3 carcinogens dealt with in the present paper are summarized in Table I. These results show: (a) that 3,4-benzpyrene is much less effective on the rabbit than on the mouse, as regards both benign and malignant tumor production; (b) that tar differs from benzpyrene in being equally effective for the two species as far as benign tumor production is concerned, though less effective for the rabbit as regards the induction of malignancy; and (c) that 9,10-dimethyl-1,2-benzanthracene differs from both tar and benzpyrene, since it is equally effective for the two species as regards both benign and malignant tumor production.

From this it would appear that the relative deficiencies in carcinogenic action of tar and of benzpyrene on the rabbit result from peculiarities of these substances, rather than from a general unresponsiveness to carcinogenesis on the part of the rabbit's skin.

SUMMARY

9,10-Dimethyl-1,2-benzanthracene is highly carcinogenic for the rabbit's skin, producing multiple, progressively growing warts after 5 weeks' application,
TABLE I: RELATIVE CARCINOGENIC POTENCY FOR THE SKIN

<table>
<thead>
<tr>
<th></th>
<th>Benign tumors</th>
<th>Malignant tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tar</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>3,4-Benzpyrene</td>
<td>+++</td>
<td>+++(+0.8)</td>
</tr>
<tr>
<td>9-10-Dimethyl-1,2-benz-</td>
<td>+++++++(+0.8)</td>
<td>++++++</td>
</tr>
<tr>
<td>antracene</td>
<td></td>
<td>+++++</td>
</tr>
</tbody>
</table>

+++ = first tumor appearing in 5 weeks or less; 
++++ = 6-15 weeks; 
+++++ = 16-25 weeks; 
++++++ = 26-35 weeks; 
+++++++ = later than the 45th week.

The values marked (a) are based on the results of Schürch (11); those marked (b) on the results of Bachmann et al. (1); all other values on the author's own results.

and malignant tumors after about 16 weeks' application.

By comparison 3,4-benzpyrene is a very weak carcinogen for the rabbit's skin, while tar, though fairly potent in the sense of inducing early warts, is relatively weak when judged on the basis of continued growth and development of malignancy.

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**ADDENDUM**

An experiment still in progress further illustrates the high carcinogenic potency of 9,10-dimethyl-1,2-benzanthracene for the rabbit's skin.

Ten rabbits were treated with a 0.5 per cent solution of this compound, application being made only once a week to the skin of the back after removal of the hair each time with electric clippers. The first papilloma appeared after 5 weeks. By the end of the eighth week tumors were already present in 6 of the 10 rabbits. In 4 of these the papillomas were single; in the other 2, they were multiple.

**REFERENCES**


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