The Sensitivity of the Skin of Hairless Mice to Chemical Carcinogenesis

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SUMMARY

The sensitivity of hairless mice to cutaneous chemical carcinogenesis has been compared with that of normal mice of the same strain with hair. A single application of 125 μg methylcholanthrene in benzene was given to 48 hairless male mice (hr/hr Oslo strain) and to 96 male mice of the same strain with hair. Among hairless mice there were 94% papilloma-bearing animals with a total of 5.9 tumors per animal after 18 months of observation, compared to 22% papilloma-bearing animals with an average of 0.3 tumors per animal among the mice with hair. The hairless mice included 31% carcinoma-bearing and 23% sarcoma-bearing animals, whereas only 1% of the mice with hair were carcinoma bearing and 3% were sarcoma bearing. Hairless mice of the hr/hr Oslo strain are thus not refractory to chemical carcinogenesis, but under the experimental conditions used in this study they are significantly more sensitive than are mice from the same strain with hair. Giovanella et al. reported almost opposite results in 1970 and came to the general conclusion that hairless mice are refractory to chemical carcinogenesis due to lack of hair follicles. Since hairless mice always have some hair follicles and rudimentary pilosebaceous appendages, comparisons between chemical carcinogenesis in hairless mice and mice with hair can neither strengthen nor weaken any theory about the hair follicle origin of epidermoid carcinomas of mouse skin.

INTRODUCTION

In 1970 Giovanella et al. (4) compared the yield of skin tumors in a strain of hairless mice with the corresponding yield in housed mice of the same strain, using the 2-stage induction-promotion technique. They found more tumors in housed than in hairless mice, and from this result they drew the general conclusions that the skin of hairless mice is relatively refractory to chemical carcinogenesis and that the hair follicles play a decisive role in mouse skin carcinogenesis.

These 2 conclusions are, in the opinion of the present authors, not correct. No strain of hairless mice is completely devoid of hair, and they all have numerous rudimentary hair follicles and pilosebaceous cysts. Hairless mice and mice with hair both have large areas of interfollicular epidermis. Comparisons of tumor yields between hairless mice and mice with hair can, therefore, neither support nor refute any hypothesis concerning hair follicle versus epidermal origin of carcinomas during chemical carcinogenesis.

Our strain of hairless (hr/hr) mice, bred since 1939 in Oslo, is very sensitive to chemical carcinogens. A single application of 125 μg MCA1 induces tumors in 90% of the animals (7, 10), and continuous treatment with a 1% solution of coal tar in benzene induces tumors in 100% of the animals (9).

In their discussion, Giovanella et al. (4) pointed to the high incidence of papillomas after chemical carcinogenesis in our hr/hr Oslo strain of mice, maintaining that our experiments lacked proper controls consisting of mice with hair from the same strain. They tended to explain our high incidence of papillomas as being due to the fact that the mice were treated at too early an age, when they still had some hairs.

The aim of the present paper is to provide the controls previously lacking and compare the effect of a single application of MCA on hairless mouse skin with the same parameter in housed mice of the same strain and sex.

MATERIALS AND METHODS

Animals. Hairless mice of the hr/hr Oslo strain were used in the experiments. Spontaneous skin tumors have not been observed in these animals, but reticuloses in internal organs are not uncommon (12). At the beginning of the experiments the animals were 70 to 80 days old, and the hairless ones were thus in a resting period of hair growth (8). The stage of the hair growth cycle in the mice with hair was not determined exactly, but these mice belonged to the same litters as the hairless ones, and histological controls of 2 animals showed a resting phase. This is of little importance, however, since shaving induces a short, complete resting phase followed by a new growth phase (11).

The hr/hr Oslo mice have been bred in our institute since 1939, and their characteristics have been thoroughly described (6, 13, 15). The animals were bred randomly, crossing housed females with hairless males. The strain is thus not purely inbred, but after about 35 years of closed colony breeding, it is rather homogeneous. Some animals are pigmented and some are not, probably due to the fact that in the 1st years of breeding mothers were taken from both the

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1 The abbreviations used are: MCA, methylcholanthrene; DMBA, 7,12-dimethyl-1,2-benzanthrene.

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1238

CANCER RESEARCH VOL. 36

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brown C3H mice and from the white WL Oslo (Kreyberg) strain.

The animals were housed in plastic cages, 8 in each box, and fed a standard diet and water ad libitum. All animals were kept in the same room. The cages were cleaned and fresh water was supplied about noon each day.

**Carcinogen Application.** Forty-eight hairless males and 96 male mice with hair were randomly selected for the experiment. The mice with hair were shaved with an electric clipper on a large area of the back skin 1 to 2 hr before carcinogen application. Exactly 0.2 ml of a solution of 1/4% MCA (S. AG, Buchs, batch No. SG 119727 40 K.) in benzene (Benzolum crystallizable pro analysi, E. Merck AG, Darmstadt, West Germany) prepared immediately before use was dropped on the anterior part of the back skin of all animals. The application was given at 12.00 Middle European Time.

**Observation of Tumor Yield.** The animals were examined every week during an observation period of 18 months (78 weeks). Each outgrowth was recorded and registered as a tumor when present for more than 2 weeks. Some animals were kept until they died; some were killed earlier because of deterioration due to a malignant tumor or other disease. Whenever possible, a necropsy was done, and at least 1 of the largest tumors from each animal was examined histologically. All lesions regarded as carcinomas or sarcomas were verified histologically. Infiltration below the musculus panniculus was used as the 1st criterion of cancer for the carcinomas.

In both groups more than 50% of the animals were alive 16 months after painting. The number of animals alive at each month of observation time is shown in Table 1.

### RESULTS

The mortality data and the tumor yields are shown in Tables 1 and 2 and in Chart 1. The tumor yield is much higher in hairless male mice than in male mice of the same strain with hair, in terms of both the percentage of animals with tumors and the number of tumors per animal. A similar difference was seen for papillomas, carcinomas, and sarcomas. A Student's t test for the data illustrated in Chart 1 shows a very significant difference in the percentage of tumor-bearing animals (p < 0.001) between hairless and haired mice treated in the same manner. All of the carcinomas originated from the skin. Of the sarcomas, 2 were

### Table 1

| Survival at following mos. after the single application of MCA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hairless mice | Animals alive | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| Tumor-bearing animals | 0 | 1 | 3 | 8 | 15 | 18 | 22 | 30 | 34 | 36 | 39 | 42 | 43 | 44 | 44 | 44 | 44 | 44 | 44 | 44 |
| % of tumor-bearing animals | 0 | 2 | 6 | 17 | 32 | 38 | 47 | 64 | 72 | 77 | 81 | 89 | 92 | 94 | 94 | 94 | 94 | 94 | 94 | 94 |
| Animals with carcinoma(s) | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 4 | 7 | 9 | 10 | 12 | 13 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| Animals with sarcoma(s) | 0 | 0 | 1 | 1 | 2 | 3 | 5 | 5 | 6 | 8 | 8 | 9 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Mice with hair | Animals alive | 96 | 94 | 94 | 94 | 93 | 91 | 89 | 89 | 88 | 86 | 83 | 80 | 79 | 79 | 78 | 77 | 73 | 64 | 24 | 0 |
| Tumor-bearing animals | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 4 | 5 | 7 | 7 | 9 | 12 | 14 | 17 | 19 | 19 | 19 | 19 | 19 |
| % of tumor-bearing animals | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 5 | 6 | 8 | 8 | 10 | 14 | 16 | 19 | 22 | 22 | 22 | 22 | 22 |
| Animals with carcinoma(s) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Animals with sarcoma(s) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* In relation to the number of animals alive at the appearance of the 1st tumor.

### Table 2

| % of animals with tumors | No. of tumors/animal* |
|---|---|---|---|---|---|---|---|---|
| Animals | Papillomas | Carcinomas | Sarcomas | Total | Papillomas | Carcinomas | Sarcomas | Total |
| Hairless mice | 94 | 31 | 23 | 94 | 5.3 | 0.5 | 0.3 | 5.9 |
| Mice with hair | 19 | 1 | 3 | 22 | 0.3 | 0.01 | 0.03 | 0.3 |

* Calculated in relation to the number of animals alive at the appearance of the 1st tumor.
U. Iversen and O. H. Iversen

reticulosarcomas of the liver, 1 was an angiosarcoma, and the others were fibrosarcomas of the corium.

DISCUSSION

When Giovanella et al. (4) compared the sensitivity of the skin of hairless mice to chemical carcinogenesis (2-stage technique with DMBA and croton oil) with the sensitivity of haired mice of the same strain, they found only 3 papillomas among 20 hairless female mice, of which 8 survived 41 weeks of treatment, and 3 papillomas among 14 male hairless mice, of which 10 survived 29 weeks of observation. In contrast, 78 papillomas and 3 sarcomas occurred in 15 male mice of the same strain with hair, of which 13 survived an observation period of 23 weeks. Their results are strikingly different from the results of the experiments reported here, where the incidence of tumor-bearing animals of the same strain is 94% in hairless mice and only 22% in mice with hair; both groups were observed for 78 weeks.

Giovanella et al. (4) discussed some factors that might explain this discrepancy, listing their own experiment and the results of some other similar studies. Those studies are all characterized by 2 features, i.e., relatively small groups of mice and an observation time of short duration. It is well known that in chemical carcinogenesis of the skin, the variation in tumor yield between small groups of mice housed in different boxes may be great (2, 3). Especially at about 20 to 30 weeks, the number of tumors per animal varies between the different boxes. The final number of tumor-bearing animals shows less variation if the observation time is long enough.

Our yield of 94% tumor-bearing animals with 5.9 tumors/animal in hairless mice is in good agreement with the tumor yield seen previously in this strain of hairless mice (7, 9, 10). As controls we used 96 male mice with hair, and our observation period was 78 weeks.

The strain of hairless mice used by Giovanella et al. (4) is not similar to ours. The hairless mice of Argonne National Laboratory have considerably more sebaceous cysts than do our hairless mice. Their mice had a high frequency of leukemia; ours have some cases of reticuloses (12).

The techniques used in the 2 studies have been different. Giovanella et al. (4) used the 2-stage induction-promotion technique with DMBA and croton oil. Croton oil is a strong promoter but also, by itself, is a weak but complete carcinogen (1, 14). "Houck's law" (5) is that dead cells never divide, and dying cells divide very slowly. It may well be that similar factors are operating in chemical carcinogenesis. One might say that dead cells never give rise to cancer, and dying cells very rarely. If this is true, and if hairless mouse epidermis is more sensitive than hairless mouse epidermis to the obvious cell killing effect of croton oil (large doses always provoke large ulcerations), then the croton oil treatment may act partly as an anticarcinogen and reduce rather than increase tumor yield in the more sensitive mice. In that case, the 2-stage technique used in hairless mice tests the cytotoxicity of croton oil and not the carcinogenesis per se. There is no experimental evidence either to prove or to disprove this possibility.

The 15 mice with hair used as controls by Giovanella et al. (4) were plucked 2 months before and once again 2 days before application of DMBA. They were thus in a short, artificially induced resting period of hair growth. Our mice with hair were shaved once with an electrical clipper 1 to 2 hr before they were given the single carcinogen application. Plucking or shaving provokes a short resting phase of hair growth before regeneration starts. Klinken-Rasmussen (11) has shown that a minimum yield of papillomas is obtained when a carcinogen is applied during a resting phase 0 to 5 days before eruption of new hairs. The fact that we used a single application in this less sensitive period in our mice with hair may partially or completely explain our low tumor yield in the control group. When Giovanella et al. (4) found many tumors in their haired mice, it may have been due to the continuous treatment with croton oil.

Continuous croton oil treatment twice weekly, such as used by Giovanella et al. (4), also keeps the treated area of the skin hairless in mice with hair. After shaving and only a single application of MCA as was done by us, the haired mice grew new hairs. This may have reduced the possibility of observing small papillomas, but the animals were carefully inspected and palpated during the study.

Based on our results, it may be concluded that we have confirmed that the hr/hr Oslo strain of hairless mice is very sensitive to cutaneous chemical carcinogenesis and that the skin of our hairless mice is much more sensitive to a single application of 125 μg MCA in benzene than is newly shaved skin of mice of the same strain with hair.

It can therefore be stated that it is not generally true that the skin of hairless mice is relatively refractory to chemical carcinogenesis and, since all hairless mice have some hair follicles, there exists no experimental evidence from hairless mice that can be used to support or refute a hypothesis about the role of the hair follicles in mouse skin carcinogenesis.

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