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7,12-DIMETHYLBENZANTHRACENE TUMOR INDUCTION IN MUTANT (HAIRLESS, ASEBIC, AND HAIRLESS-ASEBIC) MICE*

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A number of early investigations suggest that hair follicles and sebaceous glands are important in epidermal carcinogenesis. Lascassagne and Latarjet observed that tumors develop in regions of scar tissue only where hair follicles and sebaceous glands persist (1). Suntzeff *et al.* (2) failed to observe carcinomas before the development of mature hair follicles and sebaceous glands in newborn mice and also report a close correlation between the destruction of sebaceous glands and chemical carcinogeneic potency (3). In other studies, hydrocarbons have been reported to induce skin tumors without prior destruction of sebaceous glands (4, 5). These latter observations make the central role of the sebaceous gland in tumor induction less certain.

In order to better understand the role of the pilosebaceous unit in tumorigenesis, we have studied the development of carcinomas in a group of mutant mice characterized by specific defects in the pilosebaceous unit. This report is a description of the incidence, morphology, and histology of tumors induced by 7,12-dimethylbenzanthracene (DMBA) in normal, hairless, asebic, and hairless-asebic mutant stocks of mice.

MATERIALS AND METHODS

Four stocks of mice, each differing in their pilosebaceous units, were used in the present study. The normal mice were F_1 hybrid females obtained from a cross of the strains BALB/cGa and 129/RrGa. The first mutant group, homozygous for hairlessness, was obtained from the Jackson Laboratory. The second mutant group, homozygous for asebia, was obtained from the inbred colony BALB/cGa—+*ab* maintained in our laboratory (6). The third mutant group, homozygous for both hairlessness and asebia, was bred and maintained in our laboratory as described in the report by Gates, Arundell, and Karasek (7).

There were 24 mice in the normal group, 12 in the hairless, 19 in the hairless-asebic, and 17 in the asebic. All of the normal mice were females. Each of the hairless, hairless-asebic, and asebic groups of mice contained both males and females. The ages in each group ranged from 6 to 10 months. All of the animals were maintained on an ad libitum standard laboratory diet of Purina chow and water.

Each treated animal received two applications of 0.1 ml of 0.50% DMBA in acetone on the shaved midback region at a one week interval. Because of the severity of the inflammation created by 0.5% DMBA no carcinogen was applied for one week. Weekly applications of 0.1 ml of 0.25% DMBA in acetone were resumed in the fourth week of the study and were continued until the ninth week. Each treated animal received a total dose of 2500 micrograms of DMBA topically. Two control animals in each group received only placebo applications of acetone during the same time period.

Observation of the animals was continued at weekly intervals for a total of 8 months. Photographs and biopsies of representative cutaneous lesions were obtained. Autopsies were performed on mice with large invasive tumors. The tissues were fixed in buffered formalin. Sections were prepared with hematoxylin and eosin, periodic acid-Schiff, toluidine blue, colloidal iron and luxol fast blue stains (8).

RESULTS

Early Changes. Dermatitis developed in all treated animals after the first application of DMBA. The severity of the dermatitis diminished with each succeeding application of DMBA. The epidermal changes included hyperkeratosis, spongiosis, intracellular edema, and subepidermal vesicles. Edema extended into the follicles in the normal and asebic animals and into the remnants of hair follicles in the hairless and hairless-asebic mice. The sebaceous glands were destroyed in the normal and hairless mice. The dermis in all groups showed edema, focal homogenization of collagen, and an inflammatory infiltrate. Necrosis and inflammatory cells were present in the subcuta-

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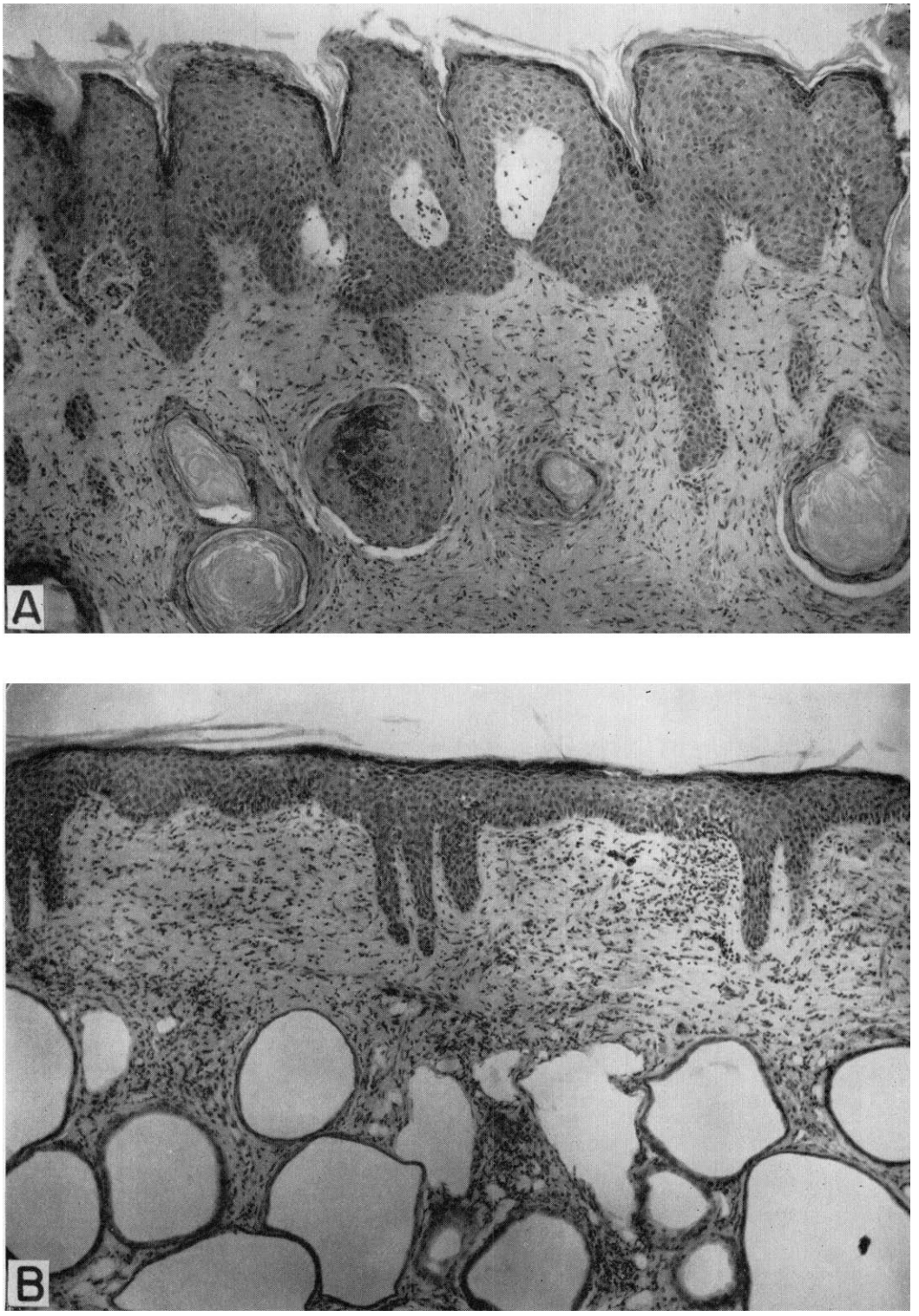

FIG. 1. Histologic changes in the skin after 3 applications of DMBA. A. Normal. B. Hairless. C. Hairless-asebic. D. Asebic. (H & E, × 103.)

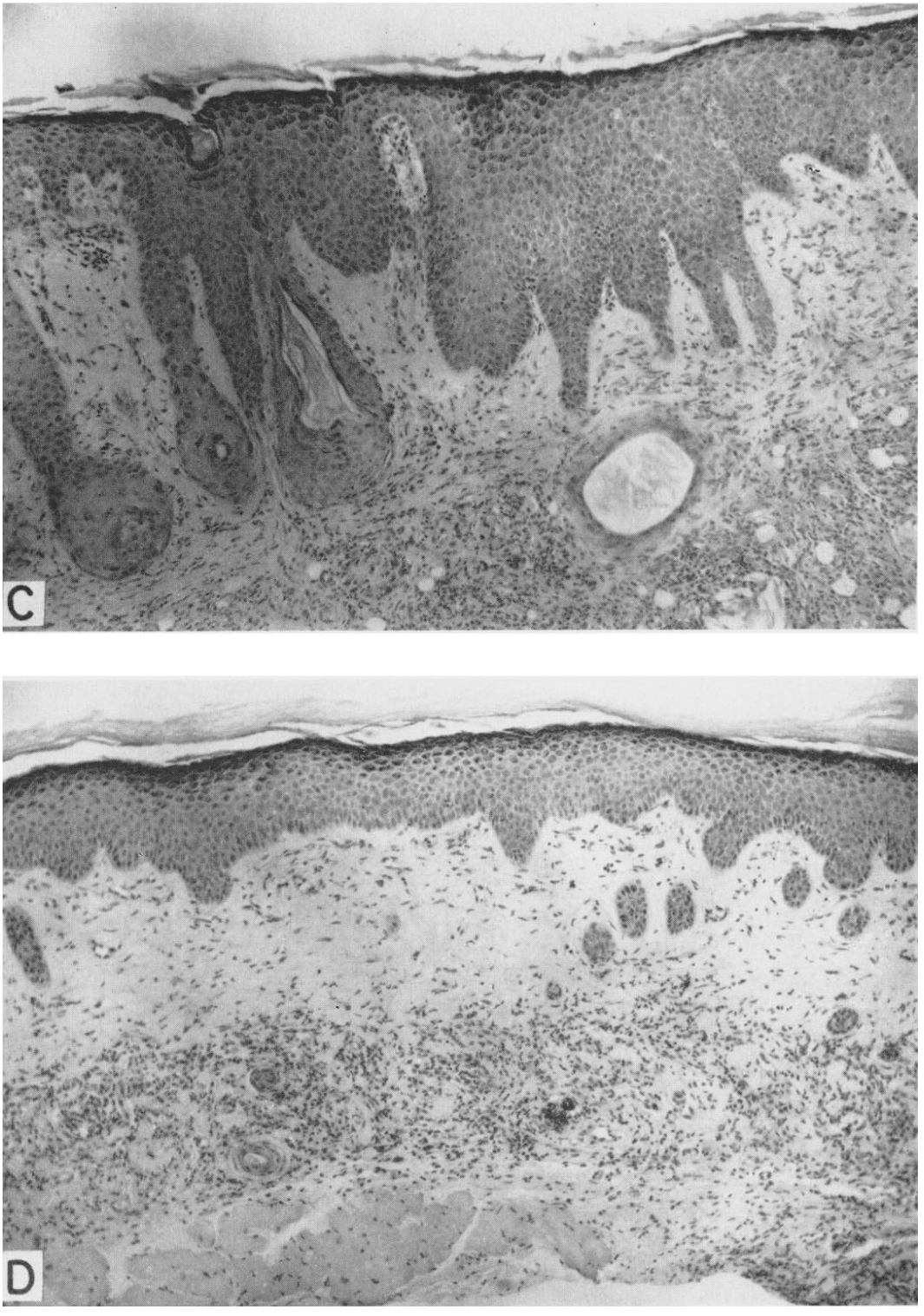

FIG. 1 C–D

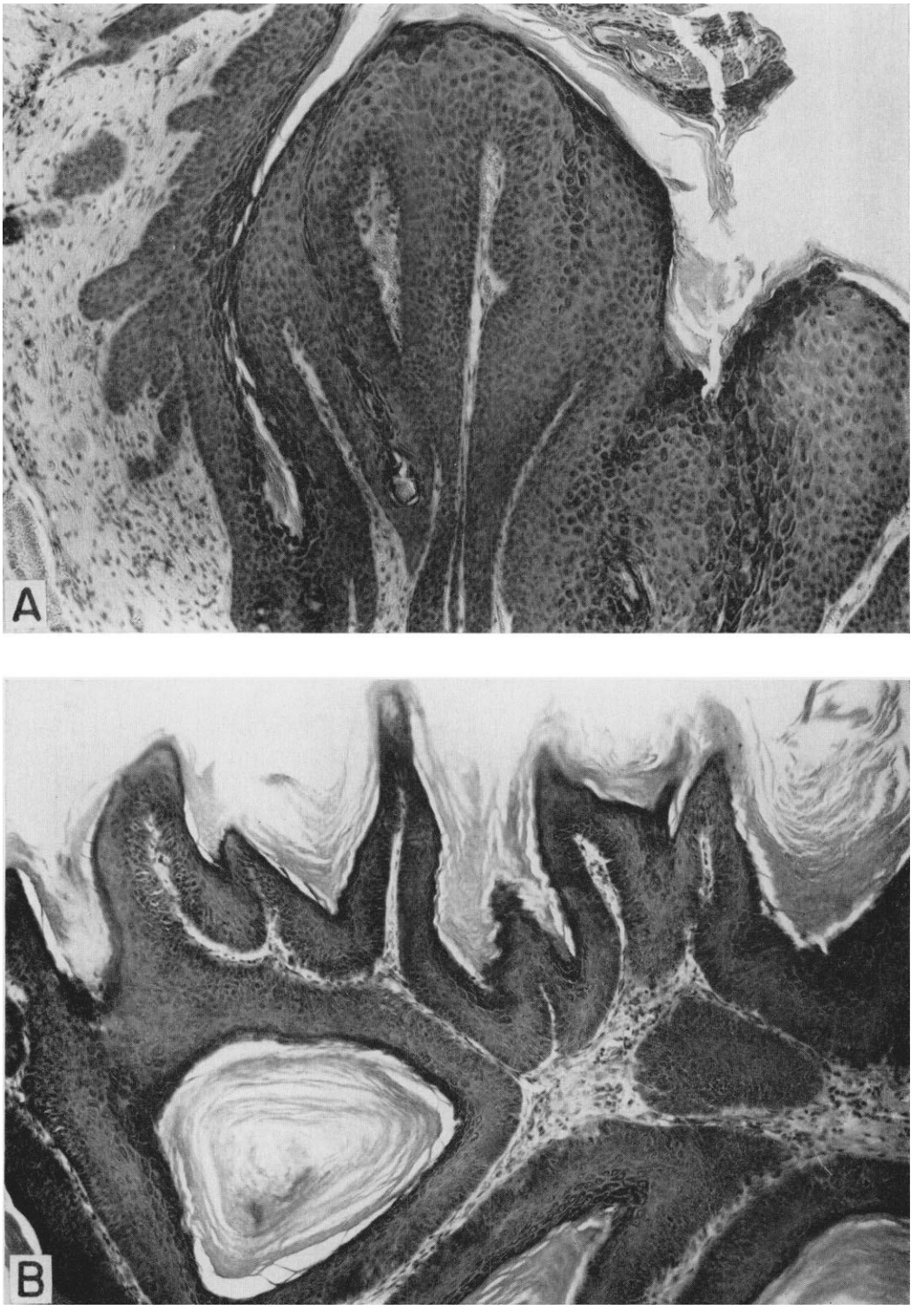

FIG. 2. Histologic changes in premalignant papillomas 5 weeks after the initial application of DMBA. A. Normal. B. Hairless. C. Hairless-asebic. D. Asebic. (H & E, × 103.)

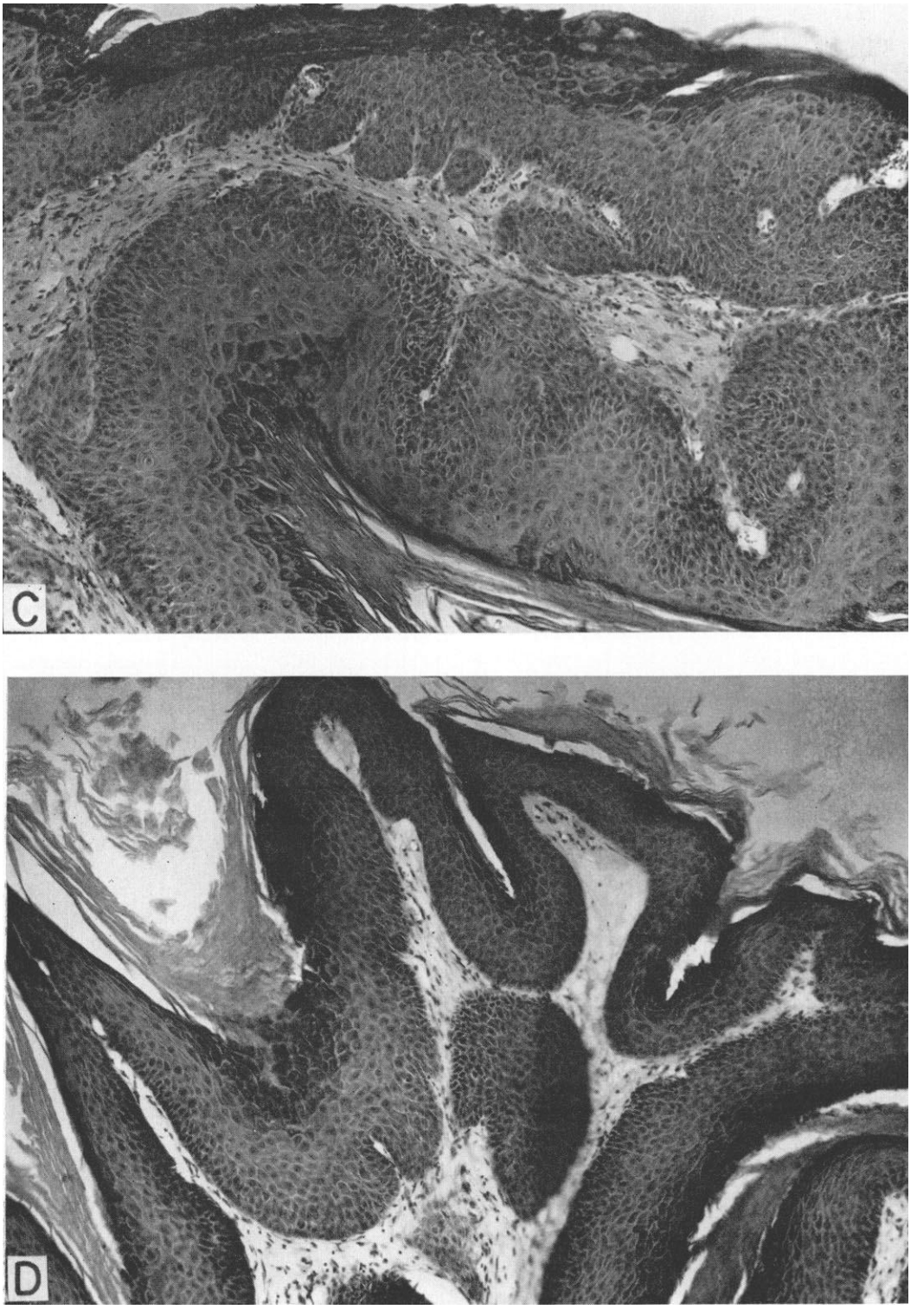

FIG. 2 C–D

TABLE I

Incidence of Squamous Cell Carcinoma

Type of mouse	Number of DMBA-treated survivors (>10 weeks)	Number of mice with squamous cell carcinoma
Normal	22	9
Hairless	10	4
Hairless-asebic	8	3
Asebic	14	3

neous fat. Special stains showed an intact basement membrane. degeneration of collagen, and an increase of acid mucopolysaccharides in the dermis and subcutaneous fat.

Pale, sclerotic plaques developed in the treated areas after the third application and persisted throughout the study. Regeneration of hair follicles in the normal and asebic mice and regeneration of sebaceous glands in the normal and hairless mice occurred in the third to fifth week of the study. The microscopic changes present in the plaques are shown in Figure 1. These changes were characterized by increasing epidermal hyperplasia and dermal fibrosis and decreasing edema and inflammatory infiltrate. Epidermal hyperplasia was more pronounced in the normal and hairless-asebic than in the hairless and asebic mice.

Premalignant Papillomas. Multiple verrucous papillomas appeared in the treated areas and in adjacent untreated areas in the fifth week. All of the DMBA-treated asebic, normal, hairless, and hairless-asebic mice developed premalignant warty papillomas by the ninth week of the study. The asebic mice developed fewer papillomas per mouse (average 7) than the other groups of mice (average 15). Spontaneous regression of the papillomas began in the third month in the asebic mice and in the seventh month in the other groups. In all groups of mice, the verrucous papillomas which persisted, coalesced to form large warty plaques. No papillomas developed in the control animals.

Microscopic changes in the verrucous papillomas from the four groups of mice are shown in Figure 2. The epidermis showed the typical premalignant changes found in warty papules induced by the topical application of carcinogens (9). Frequent mitotic figures, nuclear hyperchromasia, and intercellular separation were present in the acanthotic papillomatous lesions. In the biopsies of verrucous papillomas obtained at monthly intervals following their initial appearance, acanthosis and papillomatosis increased and replacement of large areas of the epidermis by atypical epidermal cells occurred.

Squamous Cell Carcinomas. After the third month, central ulceration and crusting occurred in the large warty plaques in each of the four groups of mice. The borders of the ulcerated areas became elevated and deeply infiltrative. Squamous cell carcinomas were noted in the third month in the normal, hairless, and hairless-asebic mice and in the fifth month in the asebic mice. All of the squamous cell carcinomas which developed arose from pre-existing papillomas. No squamous cell carcinomas developed in the control animals.

Invasion of the entire depth of the dermis and penetration of the panniculus carnosus were used as the criteria for the classification of squamous cell carcinoma. The incidence of squamous cell carcinomas in the treated mice surviving beyond the tenth week of the study is shown in Table I. Squamous cell carcinomas were found in 9 of 22 normal mice, 4 of 10 hairless mice, 3 of 8 hairless-asebic mice, and 3 of 14 asebic mice. These differences in incidence are not significant ($p < 0.10$). Although the degree of differentiation varied from tumor to tumor, there was no correlation between the degree of anaplasia and any single group of mice.

Others Tumors. In addition to squamous cell carcinomas, other benign and malignant tumors were noted in the treated animals. These tumors included two angiomas, three fibrosarcomas, and one lymphoma in normal mice; one myxosarcoma in an asebic mouse; and one lymph-angioma in a hairless mouse. No adnexal carcinomas or basal cell carcinomas developed in the treated animals during the 8 months of the study.

DISCUSSION

The three stocks of mutant mice used in the present studies show marked differences in skin metabolism and physiology. All of the mutants are retarded in growth and have a reduced fertility (6, 7). Although the adult mutants have either no hair growth (hairless,

hairless-asebic), or a greatly reduced hair growth (asebic), the skin of all of the adult mutants always shows remnants of hair follicles. The asebic mice, in addition to their specific mutations in the pilosebaceous units, are deficient in sterols esterified with long-chain fatty acids, wax esters, and wax diesters, as a result of defective fatty acid metabolism in the skin epithelial cell (10). Because of these marked differences in the skin physiology and metabolism and the unknown effects of such differences on the reactivity of the skin to carcinogens, it is surprising that each of the three mutants developed squamous cell carcinomas within 5 months after the initiation of DMBA treatment. The only detectable differences are minor changes in the degree of hyperplasia between the stocks (Fig. 1). The development of cutaneous squamous cell carcinomas in mutant mice with congenital absence of sebaceous glands indicates that sebaceous glands do not play a critical role in DMBA-induced squamous cell carcinoma.

SUMMARY

Squamous cell carcinomas were induced by topical applications of DMBA in three groups of mutant mice characterized by specific defects of the pilosebaceous units. This study shows that the presence of sebaceous glands is not necessary for DMBA-induced squamous cell carcinomas in mouse skin.

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