SUMMARY

A single 200-μg dose of tritiated 7,12-dimethylbenz(a)-anthracene containing 18 μCi tritium was applied to the skin of female hairless mice. Biopsies were taken at intervals from 30 min to 72 hr after application of the carcinogen and processed for autoradiography. All specimens showed labeling of many, but not all, dermal mast cells. The label was also found in the epidermis, hair follicles, and sebaceous glands, as previously shown by others.

INTRODUCTION

The literature dealing with experimental cutaneous chemical carcinogenesis is extensive, but few reports describe attempts to determine the histological localization of these materials in the skin.

Early workers (1, 2, 9, 10, 16, 17, 21) studied such localization by means of fluorescent microscopy after topical application of carcinogens. More recent autoradiographic work (6) described the localization of 14C-labeled DMBA3 in mouse skin and demonstrated numerous grains throughout the epidermis and dermis.

Nakai and Shubik (15) used DMBA-3H, a labeled carcinogen more suitable for histological work, and demonstrated radioautographically the localization of carcinogen in the upper part of the hair follicles, sebaceous glands, and epidermis of Swiss mice. Only occasional grains were observed in the dermis and subcutaneous tissues. Only specimens taken 24 and 48 hr after application of the carcinogen were described.

Colburn and Boutwell (7) applied 500 μCi tritiated β-propiolactone to the skin of skin-tumor-susceptible mice and took specimens for autoradiography at 2.5, 18, and 44 hr. Grains were seen in the horny layer and hair follicles. The micrograph taken by these authors of a 2.5 hr specimen shows a diffuse light labeling of the dermis.

To study the very early localization of DMBA-3H in hairless mouse skin, we performed this autoradiographic study.

MATERIALS AND METHODS

Female hairless mice 5 to 6 weeks old (HRS/J, Jackson Laboratories, Bar Harbor, Maine) weighing 15 to 18 g were used. DMBA-3H with a specific activity of 500 mCi/m mole (Nuclear-Chicago Corp. Des Plaines, Ill.) was dissolved in reagent grade acetone and diluted with unlabeled DMBA (Eastman Organic Chemicals, Rochester, N.Y.) to prepare a 0.5% DMBA-3H solution. A single dose of 200 μg DMBA-3H containing 18 μCi radioactivity was applied evenly with a micropipet to an approximately 1.5- x 1.5-sq-cm area in the interscapular region. Acetone only was applied to the skin of the rump as a control.

Two animals were sacrificed by cervical dislocation at 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 hr. Skin from the DMBA-3H-treated and acetone-treated areas was excised and processed identically. Fixation was in Karnovsky's (12) paraformaldehyde-glutaraldehyde mixture in phosphate buffer at pH 7.4, so that a portion could be used for later electron microscope studies. Tissue was dehydrated in tetrahydrofuran, embedded in paraffin, and 5-μm adjacent serial sections were cut and placed on slides. Autoradiographs were prepared by the dipping method of Messier and Leblond (13), with Kodak NTB2 emulsion. The emulsion was checked for excessive background by dipping, drying, developing, and examining microscopically a blank slide before coating tissue-containing slides. Other blank slides were dipped at the same time as tissue-containing slides and exposed in the same boxes and developed at the same time as tissue-containing slides in order to detect any build-up of excessive background during exposure. Slides were exposed for 2 weeks at 4° in 25-slot black plastic slide boxes containing Drierite and sealed with black tape. Slides were developed for 2 min in Kodak D-19 liquid developer.

1This work supported by a grant from the Milheim Foundation for Cancer Research.

2Present address: Dermatology Research Laboratories, Tufts-New England Medical Center Hospitals, 185 Harrison Ave., Boston, Mass. 02111.

3The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; DMBA-3H, tritiated 7,12-dimethylbenz(a)anthracene.

4Terracini et al. (22) have shown that a single application of 200 μg DMBA is carcinogenic in Swiss mice; whether this dose is carcinogenic in HRS/J mice as used in this study is not known.
at 20° and fixed for 2 min in Kodak F5. After washing, 1 section on each slide was stained for 30 sec with 0.02% toluidine blue to demonstrate mast cells. The entire slide was then stained for 5 min with nuclear fast red.

RESULTS

Microscopic examination or specimens taken as early as 0.5 hr (Fig. 1a) after application of DMBA-3H showed developed grains overlying cells in the dermic. By comparison of adjacent serial sections, I stained with nuclear fast red alone and the other stained with toluidine blue followed by nuclear fast red (Fig. 1c), it was evident that these grains were related almost exclusively to cells having the characteristic of mast cells; but not all mast cells contained radioactivity (compare Figs. 1b and 1c).

Grains were also found over the horny layer, especially the intrafollicular horny material, as well as over the sebaceous gland and to some extent over the follicular and interfollicular epithelium (Figs. 2 to 5).

In specimens taken at intervals up to 24 hr after application of DMBA-3H (see “Materials and Methods”), the localization of grains in general was unchanged from the earliest specimens, but they appeared more numerous in the sebaceous glands in the later specimens (compare sebaceous glands in Figs. 2 and 3). At 48 hr (Fig. 4) the well-known regressive changes in sebaceous glands (14) which occur after topical application of carcinogenic hydrocarbons were evident and by 72 hr (Fig. 5) they were virtually complete. In these specimens, as well as in the 24-hr specimen, acanthosis is present (compare Figs. 2 through 5, all at the same magnification). Aggregates of developed grains are still observed overlying mast cells. The mast cells were not degranulated, nor were any mitotic mast cells observed.

Because of the limited number of samples available in this study (2 animals/time period), no attempt was made to measure quantitatively the percentage of the mast cell population which contained radioactivity.

DISCUSSION

My investigation is most comparable to that of Nakai and Shubik (15) among those reported in the literature (1, 2, 7, 9, 10, 15–17, 21), because we used the same carcinogen, DMBA, with the same label, tritium. To some extent, our results are similar in that radioactivity was found in the epidermis, follicles, and sebaceous glands. A major difference, however, is that I found labeling of some mast cells as well. The reason for this discrepancy is not immediately apparent, but careful scrutiny of our materials and methods reveals a number of differences in experimental technique. Nakai and Shubik (15) used female Swiss mice; I used female hairless mice. Bock and Mann (5) have shown that 1 to 3 times more of a given dose of another carcinogenic hydrocarbon, benzpyrene, penetrated hairless mouse skin than penetrated the skin of their hairied sibling controls. In both studies, a single dose of 200 μg DMBA was applied to an approximately 1.5- x 1.5-sq-cm area of the interscapular skin, but I applied more radioactivity. Nakai and Shubik (15) fixed their tissue in an ethanolic fixative (Carnoy’s) and dehydrated it in absolute ethanol in which DMBA is described as “moderately soluble” (4), while I used an aqueous formaldehyde-glutaraldehyde fixative (12) in which DMBA is not soluble (14), but used tetrahydrofuran for dehydration in which DMBA is markedly soluble. Finally, different emulsions and exposure times were used. It is possible that any of the above differences in technique alone or in combination may be responsible for our discordant results, but the increased penetrability of hairless mouse skin coupled with a higher dose of radioactivity seems the most likely cause.

It is likely that the dermal cells in which the radioactive label is localized are mast cells, since the label is found almost entirely over cells whose granules take the toluidine blue stain. Not more than one-half of the mast cells in a given low-power field contain radioactive label. One must bear in mind that at about 3 weeks of age hairless mice normally develop a dermal inflammatory infiltrate coincident with hair bulb degeneration and this infiltrate persists for the rest of their lives (11). It is conceivable, therefore, that the cells containing toluidine blue-staining granules and radioactivity could be macrophages which have separately engulfed labeled carcinogen, as well as granules from degranulated mast cells. This interpretation seems unlikely, however, since the mast cells did not appear degranulated by light microscopy and since preliminary ultrastructural (non-autoradiographic) studies of tissue taken 30 and 60 min after application of DMBA show that the mast cell granules are in general quite intact and are within mast cells.

One can only speculate with regard to the implications of the localization of a topically applied carcinogen in mast cells. An increased number of mast cells in the dermis of carcinogen-treated skin has been recognized for many years (8), but the significance of this finding is not known. Among the roles postulated for this mast cell reaction is defense against (8) and promotion of (18) neoplasia. My findings cannot settle this issue, but they indicate at least that there is a direct interaction between the carcinogen, DMBA, and the mast cell. Uptake of DMBA by mast cells may play some part in the increased uptake of tritiated thymidine (3) and the stimulation of mitotic activity (19) of mast cells which has been shown to occur in DMBA-treated mouse skin.

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5This comparison method of examination was necessary because with the fixative used the mast cell granules stained orthochromatically dark blue (20) with toluidine blue. It was often quite difficult to decide whether black autoradiographic grains were also present in a toluidine blue-stained cell despite the different levels of focus of mast cell granules and developed grains.

6DMBA (50 mg) was soluble in 0.3 ml tetrahydrofuran at 22°. The same quantity of DMBA required 30 ml absolute ethanol to dissolve completely.
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REFERENCES
Fig. 1. At 0.5 hr after application of DMBA-^H, a, low-magnification survey autoradiograph to show the general disposition of labeled dermal cells (circles). The box outlines the area shown at higher magnification in b. Nuclear fast red, X 240. b, higher magnification of a portion of a showing 2 labeled dermal cells (A and B). Nuclear fast red, X 480. c, corresponding area of a section adjacent to that shown in b, but stained with toluidine blue, followed by nuclear fast red. Two mast cells, A and B', correspond to the labeled cells A and B shown in b. Five other mast cells without radioactive labeling are present. X 480.
Fig. 2. At 1 hr. The horny layer and plug is heavily labeled. Labeled dermal cells are present. Note sparse label in follicular epithelium and sebaceous glands, compared with succeeding figures. Nuclear fast red, X 280.

Fig. 3. At 24 hr. Increased labeling of sebaceous glands and epithelium. A labeled dermal cell is shown. Nuclear fast red, X 280.

Fig. 4. At 48 hr. Epithelium clearly thickened. The sebaceous glands are regressing. Labeled dermal cells are present. Nuclear fast red, X 280.

Fig. 5. At 72 hr. The sebaceous glands have regressed, leaving only a thin rim of epithelium. The follicular canals contain labeled horny material. Labeled dermal cells are present. Nuclear fast red, X 280.
Autoradiographic Localization of Tritiated 7,12-Dimethylbenz(a)anthracene in Mast Cells of Hairless Mouse Skin

William M. Tarnowski


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